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<p>Figure A: Purification gel showing a band at 919 kDa. Figure B: Western blot showing a band at 919 kDa. Figure C: Flow cytometry histogram showing a peak at 919 kDa. Figure D: Bactericidal assay graph showing log10 CFU over time for preimmune, GST, and 919.</p>			
(57) Abstract			
<p>The invention provides methods of obtaining immunogenic proteins from genomic sequences including <i>Neisseria</i>, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of <i>Neisseria meningitidis B</i>. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.</p>			

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## NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

## BACKGROUND

*Neisseria meningitidis* is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

*N. meningitidis* causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines*, *supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of  $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention to provide Neisserial DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

- 4 -

Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

## THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See [ftp://ftp.tigr.org/pub/data/n\\_meningitidis](ftp://ftp.tigr.org/pub/data/n_meningitidis) on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

- 6 -

greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least  $n$  consecutive nucleotides from the sequences and, depending on the particular sequence,  $n$  is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.



- 7 -

The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydrophathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psорт.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or

- 10 -

strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

- 11 -

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

#### Methodology - Summary of standard procedures and techniques.

##### General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and II* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference.

#### Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

##### i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

- 14 -

mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection



- 15 -

(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wiersel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, *Gibberellins*: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not

- 16 -

readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmsink and Dons, 1993, *Plant Mol. Biol. Reptr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,

*Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

### iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

- 19 -

homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

- 20 -

into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha)  $\alpha$ -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15  $\mu\text{m}$  in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.



iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nuc. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberg)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

- 25 -

fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the

coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with  $\text{CaCl}_2$  or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

- 28 -

Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

#### v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

- 29 -

either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

- 30 -

be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCI/1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be



either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guilliermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

## Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).

## Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200  $\mu$ g/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this

- 35 -

invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

- 36 -

labels into distinct classes, as the same label may serve in several different modes. For example, <sup>125</sup>I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with <sup>125</sup>I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

#### Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

- 37 -

advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

#### Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

- 38 -

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

### Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a



larger particle size emulsion, and (c) Ribi<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox<sup>TM</sup>); (3) saponin adjuvants, such as Stimulon<sup>TM</sup> (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

#### Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No1 VR-590), Kirsten, Harvey Sarcoma Virus and

Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

- 45 -

ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

- 46 -

further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.



### Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hypodermic sprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

### Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

#### A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

- 48 -

granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

#### E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These

lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines; human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as  $\Phi$ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

### Immunodiagnostic Assays

*Neisseria* MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

### Nucleic Acid Hybridization

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated  $T_m$  of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to  $10^{-9}$  to  $10^{-8}$  g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of  $10^8$  cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than  $10^8$  cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature ( $T_m$ ) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4(\%(G + C)) - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch})$$

where  $C_i$  is the salt concentration (monovalent ions) and  $n$  is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

#### Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so



a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al.* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

## EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (e.g. to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [see also R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et al., Nature 390, 580-586 (1997); J.-F. Tomb, et al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

#### Chromosomal DNA Preparation

*N. meningitidis* strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were

- 58 -

lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one  $\text{CHCl}_3$ /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

### Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGGATCCCATATG</u>	( <i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	( <i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	( <i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	( <i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCATATGGCCATGG</u>	( <i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	( <i>Bam</i> HI)

Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (NheI)

3'-end primer tail: CGGGATCC (BamHI)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml NH<sub>4</sub>-OH, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1ml of water. OD<sub>260</sub> was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/µl.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC.

### Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 µM of each oligo, 400-800 µM dNTPs solution, 1x PCR

- 60 -

buffer (including 1.5 mM MgCl<sub>2</sub>), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

### Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

NdeI/XhoI or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

BamHI/XhoI or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

#### **Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)**

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD<sub>260</sub> of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

#### **Cloning**

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

In order to introduce the recombinant plasmid in a suitable strain, 100  $\mu$ l *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800  $\mu$ l LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200  $\mu$ l of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100  $\mu$ g/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30  $\mu$ l. 5  $\mu$ l of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

### **Cloning**

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E.coli* host W3110.

### **Expression**

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1  $\mu$ l of each construct was used to transform 30  $\mu$ l of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100  $\mu$ g/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100  $\mu$ g/ml) in 100 ml flasks, making sure that the OD<sub>600</sub> ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for



pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

#### **GST-fusion proteins large-scale purification.**

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD<sub>280</sub> of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer 10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M'') (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

**His-fusion soluble proteins large-scale purification.**

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>350</sub> 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150μl Ni<sup>2+</sup>-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The His-fusion protein was eluted by addition of 700μl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D<sub>280</sub> was 0.1. 21μl of each fraction were loaded on a 12% SDS gel.

**His-fusion insoluble proteins large-scale purification.**

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D<sub>550</sub> 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

and thawed twice and centrifuged again. The supernatant was stored at -20°C, while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150µl Ni<sup>2+</sup>-resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C. The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD<sub>280</sub> of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

#### His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

#### Mice immunisations

20µg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with Al(OH)<sub>3</sub> as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other

- 66 -

ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

#### **ELISA assay (sera analysis)**

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H<sub>2</sub>O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA was considered positive when OD<sub>490</sub> was 2.5 times the respective pre-immune sera.

#### **FACScan bacteria Binding Assay procedure.**

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

- 67 -

OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN<sub>3</sub>) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD<sub>620</sub> of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)<sub>2</sub> goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Threshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

### **OMV preparations**

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice ( 50% duty cycle, 50% output ). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

**Whole Extracts preparation**

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

**Western blotting**

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

**Bactericidal assay**

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD<sub>620</sub> was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD<sub>620</sub> of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

- 69 -

Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

### EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

#### Localization of the ORFs

ORF:            contig:

279            gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:  
m279.seq

```

1   ATACGCGGA  TTTGCGGCTG  CTTGATTCCA  ACGGTTTCCA  GGGCTTCGCG
51  AAGTTTGTGCG  GCGGCGGGTT  TCATCAGGCT  GCAATGGGAA  GGTACGGACA
101 CGGCGAGCGG  CAGGCGCGGT  TTGSCACCGG  CTTCTTTGGC  GGCAGCCATG
151 GCGCGTCCGA  CGGCGCGGCG  GTTGCGCTGCA  ATCAGCATTT  GTCCGGGTGA
201 GTTGAAGTTG  ACGGCTTCGA  CCACTTCGCT  TTGGCGGGCT  TCGGCACAAA
251 TGGCTTTAAC  CTGCTCATCT  TCCAAGCCGA  GAATCGCCGC  CATTGCGCCC
301 ACGCCTTGCG  GTACGGCGGA  CTGCATCAGT  TCGGCGCGCA  GCGCACGAG
351 TTTGACCGCG  TCGCAAAAT  TCAATGCGCC  GCGGCAACG  AGTGGGTGT
401 ATTGCGCGAG  CTGTGTCCG  GCAACGCGG  CAGGCGTTTT  GCGCGCGCT
451 TCTAAATAG

```

- 70 -

This corresponds to the amino acid sequence <SEQ ID 3; ORF 279>:

m279.pep

```

1  ITRICGCLIS TVFRASASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51  ARPTAALPA ITICPGELKL TASTTSLWAA SAQMALTCSS SKPRIAIAIP
101 TPGGTADCS SARRRTSLTA SAKFNAPAAAT SAVYSPRLCP ATAAGVLPPA
151 SK*

```

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 4>:

g279.seq

```

1  atgacgcgga tttgcggctg cttgatttca acggttttga gtgtttccgc
51  aagttttctg gcggcgggtt tcatcaggct gcaatgggaa ggaacggata
101 ccggcagcgg caggcgcggt ttggctccgg cttctttggc ggcagccatg
151' tgcgctccga cggcgggcgg gttgcctgca atcacgactt gtccgggcga
201 gtgaagttg acggcttcga ccaactcgcc ctgtgcggat tcggcacaaa
251 tctgcctgac ctgttcatct tccaaaccca aaatggcggc cattgcgcct
301 acgccttgcg gtacggcgga ctgcatcagt tcggcgcgca ggcggacgag
351 tttagcggca tcggcaaaat ccaatgtctt ggccggcgca agcggcgtgt
401 attcgccgag gctgtgtcgg gcaacggcgg caggcggttt gccgccact
451 tccaaatag

```

This corresponds to the amino acid sequence <SEQ ID 5; ORF 279.ng>:

g279.pep

```

1  MTRICGCLIS TVLSVSASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51  VRPTAALPA ITTCPGELKL TASTTSPCAD SAQICLTCSS SKPRMAIAP
101 TPGGTADCS SARRRTSLTA SAKSNASAAAT SAVYSPRLCP ATAAGVLFFT
151 SK*

```

ORF 279 shows 89.5% identity over a 152 aa overlap with a predicted ORF (ORF 279.ng) from *N. gonorrhoeae*:

m279.pep	ITRICGLISTVFRASASLSAAGFIRLOWEGTDTGSGRARLAPASLAAAMARPTAALPA	10	20	30	40	50	60
g279	MTRICGCLISTVLSVSASLSAAGFIRLOWEGTDTGSGRARLAPASLAAAMVRPTAALPA	10	20	30	40	50	60
m279.pep	ITICPGELKLTASTTSLWAAASQMALTCSSSKPRIAIAIPTPGGTADCSARRRTSLTA	70	80	90	100	110	120
g279	ITTCPGELKLTASTTSPCADSAQICLTCSSSKPRMAIAPTPGGTADCSARRRTSLTA	70	80	90	100	110	120
m279.pep	SAKFNAPAAATSAVYSPRLCPATAAGVLPPASKK	130	140	150			
g279	SAKSNASAAATSAVYSPRLCPATAAGVLFFTSSK	130	140	150			

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 6>:

a279.seq

```

1  ATGACNCNGA TTTGCGGGCTG CTTGATTTC ACGGTTTNN A GGGCTTCGGC
51  GAGTTTGTGC GCGGCGGGTT TCATGAGGCT GCAATGGGA GGTACNGACA
101 CNGGCAGCGG CAGGGCGCGT TTGGCGCGGG CTTCTTGGC GGAAGCATA
151 GCGCGCTCGA CCGCGGGCGG ATTGCTTCA TCACACGACT GTCCGGCGCA
201 GTTGAAGTTG ACGGCTTCAA CCACTTCAT CTGTGCGGAT TCGGCGCAAA
251 TTTGTTTTC CTTGTTTCA TCACACGCGA GAATGCGCGC CATTGCGGCC
301 ACGGCTTCGG GTACGGCGGA CTGCATCAGT TCGGCGCGCA NGCGACGAG
351 TTTGACCGCG TCGGCAAAAT CCAATGCGCC GCGCGCAAC AGTGCAGTGT

```



- 71 -

401 ATTCGCCGAN GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCGCT  
451 TCCGAATAG

This corresponds to the amino acid sequence <SEQ ID 7; ORF 279.a>:

a279.pep  
1 MTXICGCLIS TVXRASASLS AAGFMRLQWE GTDTGSGRAR LAPASLAASI  
51 ARSTAAALPA ITTCPGELKL TASTTSSCAD SAQICFTCSS SKPRIAIAIP  
101 TPCGTADCLIS SARXRTSLTA SAKSNAPAAT SAVYSPXLCP ATAAGVLPPA  
151 SE\*

m279/a279 ORFs 279 and 279.a showed a 88.2% identity in 152 aa overlap

	10	20	30	40	50	60
m279.pep	ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRARLAPASLAAMARPTAAALPA					
a279	MTXICGCLISTVXRASASLSAAGFMRLQWEGTDTGSGRARLAPASLAASIAARSTAAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITICPGELKLTAATSTSLWAASQAQALTCSKSPRIAAIATPCGTADCLISSARRRTSLTA					
a279	ITTCPGELKLTAATSTSSCADSAQICFTCSSKSPRIAAIATPCGTADCLISSARXRTSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKENAPAATSAVYSPRLCPATAAGVLPPASKX					
a279	SAKSNAPAATSAVYSPXLCPATAAGVLPPASEX					
	130	140	150			

519 and 519-1 gnm7.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 8>:

m519.seq (partial)  
1 ..TCCGTTATCG GCGTATGGA GTTGACAAA ACGTTTGAAG AACGCGACGA  
51 AATCAACAGT ACTGTTGTTG CGGCTTTGGA CGAGCGCGCC GGCGTTTGG  
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGGTTCC GCGCGAAGAA  
151 ATCCTTCGCT CAATGCGAGC GCAAAATTACT GCCGAACGCG AAAAAACGCGC  
201 CCGTATCGCC GAATCGGAAG TCGTAAAAAT CGAACAAATC AACCTTGCCA  
251 GTGGTCAGCG CGAAGCGGAA ATCCAAACAAT CCGAAGCGCA GGCTCAGGCT  
301 GCGGTCAATG CGTCAAAATGCG CGAGAAAATC GCCCGCATCA ACGCGGCCAA  
351 AGGTGAAGCG GAATCCTTGC GCCTTGTGTC CGAAGCCAAT GCGGAAAGCCA  
401 TCCGTCAAAT TGCGCGCGCC CTTCAAACCC AAGCGCGTGC GGATGCGGTC  
451 AATCTGAAGA TTGCGGAACA ATACGTGCTG CGGTCAACA ATCTTGCCAA  
501 AGAAGCAAT ACGCTGATTA TGCCCGCCAA TGTTCGCGAC ATCGGCAGCC  
551 TGATTCTGCG CGGTATGAAA ATTATCGACA CGACCAAAAC CGCCAAATAA

This corresponds to the amino acid sequence <SEQ ID 9; ORF 519>:

m519.pep (partial)  
1 ..SVIGRMELDK TPERDEINS TVVAALDEAA GANGVXVLRV EIKDLVPPQE  
51 ILKSMQAQIT ASKEKRARIA ESEGRKIEQI NLASGGREAE IQQSEGEADA  
101 AVNASNAEKI ARINRAKGEA ESLRLVAEAN AEAIRQIAAA LQTQGGADAV  
151 NLKIAEQYVA APNNLAKESN TLIMPANVAD IGSLSAGMK IIDSSTKAK\*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 10>:

g519.seq  
1 atggaatttt tcaatatctt gttggcagcc gtcgccgttt tcggcttcaa  
51 atcctttgtc gtcaccccc agcaggaagt ccacgttgtc gaaaggtctg

- 72 -

```

101 ggcgtttcca tcgcgccttg acggccggtt tgaatatatt gattcccttt
151 atcgaccgcg tcgcctacgc ccattcgctg aaagaaatcc ctttagacgt
201 acccagccag gtctgcgatca cgcgcgataa tacgcaattg actgttgacg
251 gcatcatcta ttcccaagta accgatccca aactcgctct atacggttcg
301 agcaactaca ttatggcaat tacccagcgt gcccaaacga cgtcgcgttc
351 cgttatcggg cgtatggagt tggacaaaac gtttgaagaa cgcgcagaaa
401 tcaacagtac cgtcgtctcc gccctcgatg aagcgcgcgg ggcttggggg
451 gtgaagaagtc tcggttacga aatcaaggat ttggttcgcg cgcaagaaat
501 ctttcgcgca atcgaggcac aaattaccgc cgaacgcgaa aaacgcgcgc
551 gtattgcgca atccgaaggc cgtaaaatcg aacaaatcaa cttgcccagt
601 ggtagcgcgt aagccgaagt ccaacaatcc gaaggcgagg ctacggctgc
651 ggtaaatgct tccaatgcgc agaaaatcgc cgcgatcaac cgcgcgaag
701 gcgaagcgga atccctgcgc cttgttgccg aagccaatgc cgaagccaac
751 cgtcaaatg cgcgcgcctt tcaaacccaa agcggggcgg atgcggtcaa
801 tctgaagatt gcgggacaat acgttaccgc gttcaaaaat cttgccaag
851 aagacaatgc cgggattaag ccgcgcaagg ttgcgcaaat cgggaacct
901 aattttcggc ggcatgaaaa attttcgcca gaagcaaaaa cgcgcaata
951 a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

```

g519.pep
1  MEFFILLAA VAVFGFKSFV VIPQQEVHVV ERLGRFHERL TAGNLINILFP
51  IDRVAYRHSL KEIFLDVPSQ VCITRDNTQL TVDGIYPOV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVRLYEIKD LVPPQELIRA MQAQITAERE KHARIESSE RKIEQINLAS
201 GQRBAEIQOS EGEEAQAQVNA SNAEKIARIN RAKGEASRL LVAEANABAN
251 RQIAAALQTQ SGADAVNLKI AGQYVTAFFN LAKEDNTRIK PAKVAEIGNP
301 NFRRHEKFSF EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

m519/g519

```

                                     10      20      30
m519.pep                               SVIGRMELDKTFEERDEINSTVVAALDEAA
g519  YFQVTDPKLASVYSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
      90      100     110     120     130     140

                                     40      50      60      70      80      90
m519.pep  GAWGVKVLRYEIKDLVPPQEILRSMQQAITAEREKRARIAESSEGRKIEQINLASGQREAE
g519  GAWGVKVLRYEIKDLVPPQEILRAMQAQITAEREKRARIAESSEGRKIEQINLASGQREAE
      150     160     170     180     190     200

                                     100     110     120     130     140     150
m519.pep  IQQSEGEAQAQVNASNAEKIARINRAKGEASRLVVAEANAIAIRQIAAALQTQGGADAV
g519  IQQSEGEAQAQVNASNAEKIARINRAKGEASRLVVAEANAIAIRQIAAALQTQSGADAV
      210     220     230     240     250     260

                                     160     170     180     190     200
m519.pep  NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL-ISAQMKIIDSSKTAK
g519  NLKIAEQYVTAFFNKLAKEDNTRIKPAKVAEIGNPNFRRHEKFSPEAKTAK
      270     280     290     300     310

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq

- 73 -

```

1  ATGGAATTTT  TCAATTATCTT  GCTGGCAGCC  GTCGTTGTTT  TCGGCTTCAA
51  ATCCTTTTGT  GTCATCCCCAC  AGCAGGAAGT  CCACGTTGTC  GAAAGGCTCG
101  GGCCTTTTCA  TCGCGCCCTG  ACGGCCCGTT  TGAATATTTT  GATTTCCTTT
151  ATCGAGCCGG  TCGCGTACCG  CCATTGCGTG  AAAGAATCC  CTTTAGCGGT
201  ACCGAGCGAG  GCTCTGCATCA  CGCGACGCA  TACGACGCTG  ACTGTTGAG
251  GTATCATCTA  TTTCCAACTA  ACCGACCCCA  AACTCGCTTC  ATACGGTTTC
301  AGCAACTACA  TTATGGGGAT  TACCAGCTT  GCCCAAAGCA  CGCTCGCTTC
351  CGTTATCGGG  CGTATGGAAT  TGGCAAAAC  GTTTGAAGAA  CGCGACGAAA
401  TCAACAGCAC  CGTCGTCTCC  GCCTTCGATG  AAGCCGCGGG  AGCTTGGGGT
451  GTGAAGGTTT  TGCGTTATGA  GATTAAAGAC  TTGTTTCCGC  CGCAAGAAAT
501  CCTTCGCTCA  ATCGAGCGCG  AATTACTGCG  TGAACGGGAA  AAACGCGCCC
551  GTATCGCCGA  ATCCGAAGGT  CGTAAATCG  AACAATCAA  CCTTGCAGAT
601  GGTGAGCGCG  AAGCGGAAAT  CCAACAATCC  GAAGGCGAGG  CTCAGGCTGC
651  GGTCAATGCG  TCAATGCGCG  AGAAATTCGC  CGCATCAAC  CGCGCCAAAG
701  GTGAAGCGGA  ATCCTTGGCG  CTTGTTGCG  AAGCCANTGC  CGAAGCCATC
751  CGTCAAATTC  CGCGCGCCCT  TCAAAACCAA  GCGCGTGGCG  ATGCGGTCAA
801  TCTGAAGATT  GCGGAACAAT  ACGTCGCGCG  GTTCAACAAT  CTTGCCAAAG
851  AAGCAATATC  GGTGATTATG  CCGCGCAATG  TTGCCGACAT  CGGCAGCTCG
901  ATTTCTGCGG  GTATGAAAT  TATCGACAGC  AGCAAAACCG  CCAATAAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1  MEFFIILLAA  VVVGFPKSFV  VIPQQEVHV  ERLGRFHRAL  TAGLINILIP
51  IDRVAIRHSL  KEIFLDVPSQ  VCITRDNTQL  TVDGIIFYQV  TDPKLASYGS
101  SNYIMAITQL  AQTTLRVIG  RMELDKTFEE  RDEINSTVVS  ALDEAAGAWG
151  VKVLRYEIKD  LVPPQEILRS  MQAQITAERE  KRARIAESEG  RKIEQINLAS
201  GQREAEIQQS  EGEAQAVNA  SNAEKIARIN  RAKGEAESLR  LVAEANAIAI
251  RIQAALQTO  GGAADVNLKI  AEQYVAFPNN  LAKESNTLIM  PANVADIGSL
301  ISAGMKIIDS  SKTAK*

m519/a519  ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

10          20          30
m519.pep          SVIGRMELDKTFEERDEINSTVVAALDEAA
a519              YFQVTDPKLASYSSSNYIMAITQLAQTTLRVIGRMELDKTFEERDEINSTVVSALDEAA
90          100         110         120         130         140

40          50          60          70          80          90
m519.pep          GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
a519              GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
150         160         170         180         190         200

100         110         120         130         140         150
m519.pep          TQSEGEQAQAAVNASNAEKIARINRAKGEAESLR LVAEANAIAIRQIAALQTQGGADRV
a519              TQSEGEQAQAAVNASNAEKIARINRAKGEAESLR LVAEANAIAIRQIAALQTQGGADAV
210         220         230         240         250         260

160         170         180         190         200
m519.pep          NLKIAEQYVAAPFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
a519              NLKIAEQYVAAPFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
270         280         290         300         310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq

- 74 -

```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTCGCCGTTT TCGGTTTCAA
51  ATCCTTTTGTG GTCATCCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTGG
101  GCGGCTTTCCA TCGGCGCCCTG ACGGCGCGTT TGAATATTTT GATTCCCTTT
151  ATCGACCGCG TCGGCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201  ACCGAGCCAG GTCTGCATCA CGCGCGACAA TACGACGCTG ACTGTTGAGG
251  GCATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTGG
301  AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAACGA CGCTGCGTTC
351  CGTTATCGGG CGTATGGAGT TGGCAAAAC GTTTGAAGAA CGCGACGAAA
401  TCAACAGTAC TGTTGTTGCG GTTTTGACG AGGCGCGCGG GCGTTGGGTT
451  GTGAAGGTTT TGCCTTATGA GATTAAAGAC TTGGTTCGCG CGCAAGAAAT
501  CCTTCGCTCA ATGCAGCGCG AATTACTGCG CGAAGCGCAA AAACGCGCCC
551  GTATCGCCGA ATCCGAAGGT CGTAAATTCG AACAAATCAA CCTTGCAGT
601  GGTGAGCGCG AAGCGCAAAAT CCACACATCC GAAGCGGAGG CTCAGGCTGC
651  GGTCAATGCG TCAAAATGCG AGAAATTCG CGCATCAAC CGCGCCAAAG
701  GTGAAGCGGA ATCCTTGCGC CTTGTTGCGG AAGCCAAATG CGAAGCCATC
751  CGTCAAATTT CGCGCGCCCT TCAAACCCAA GCGGGTGGCG ATGCGGTCAA
801  TCTGAAGATT GCGGAACAAAT ACGTCGCTGC GTTCAACAA CTGCGCAAG
851  AAAGCAATAC GCTGATTATG CCGGCCAATG TTGCGGACAT CGGCAGCCTG
901  ATTTCTGCGG GTATGAAAT TATCGACAGC AGCAAAACCG CCAATATA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVPGFKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHS L KEIFLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLAYSYS
101  SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151  VKVLRYEIKD LVPPQELIRS MQAQITAEER KRARIAESEG RKIEQINLAS
201  GQREAEIQQS EGEAQAAYNA SNAEKIARIN RAKGEAESLR LVAEANAEAI
251  RQIAAALQTQ GGADAVNLKI AEQYVAFNN LAKESNTLIM PANVADIGSL
301  ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTCGCCGTTT TCGGCTTCAA
51  ATCCTTTTGTG GTCATCCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101  GCGGCTTTCCA TCGGCGCCCTG ACGGCGCGTT TGAATATTTT GATTCCCTTT
151  ATCGACCGCG TCGGCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201  ACCGAGCCAG GTCTGCATCA CGCGCGATAA TACGCAATG ACTGTTGACG
251  GCATCATCTA TTTCCAAGTA ACCGATCCCA AACTCGCCTC ATACGGTTGG
301  AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAACGA CGCTGCGTTC
351  CGTTATCGGG CGTATGGAGT TGGCAAAAC GTTTGAAGAA CGCGACGAAA
401  TCAACAGTAC CGTCTCTCC CGCCTCGATG AAGCGCGCGG GCGTTGGGTT
451  GTGAAGTCC TCGGTTACGA AATCAAGGAT TTGGTTCGCG CGCAAGAAAT
501  CCTTCGCGCA ATGCAGGCAC AATTACGCG CGAAGCGGAA AAACGCGCCC
551  GTATTGCCGA ATCCGAAGGC CGTAAATTCG AACAAATCAA CCTTGCAGT
601  GGTGAGCGCG AAGCGCAAAAT CCACACATCC GAAGCGGAGG CTCAGGCTGC
651  GGTCAATGCG TCAAAATGCG AGAAATTCG CGCATCAAC CGCGCCAAAG
701  GCGAAGCGGA ATCCTTGCGC CTTGTTGCGG AAGCCAAATG CGAAGCCATC
751  CGTCAAATTT CGCGCGCCCT TCAAACCCAA GCGGGGCGCG ATGCGGTCAA
801  TCTGAAGATT GCGGAACAAAT ACGTAGCCGC GTTCAACAA CTGCGCAAG
851  AAAGCAATAC GCTGATTATG CCGGCCAATG TTGCGGACAT CGGCAGCCTG
901  ATTTCTGCGG GCATGAAAT TATCGACAGC AGCAAAACCG CCAATATA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVPGFKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHS L KEIFLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLAYSYS
101  SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151  VKVLRYEIKD LVPPQELIRS MQAQITAEER KRARIAESEG RKIEQINLAS
201  GQREAEIQQS EGEAQAAYNA SNAEKIARIN RAKGEAESLR LVAEANAEAI
251  RQIAAALQTQ GGADAVNLKI AEQYVAFNN LAKESNTLIM PANVADIGSL
301  ISAGMKIIDS SKTAK*

```

- 75 -

m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

```

      10      20      30      40      50      60
g519-1.pep MEFFIILIAAVAVFGFKSPVVI PQQEVHVVERLGRFHALTAGLNILIPFIDRVAYRHSL
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      MEFFIILLVAVAVFGFKSPVVI PQQEVHVVERLGRFHALTAGLNILIPFIDRVAYRHSL
      10      20      30      40      50      60

      70      80      90      100     110     120
g519-1.pep KEIPLDVPSQVCITRNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITOLAQTTLRSVIG
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      KEIPLDVPSQVCITRNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITOLAQTTLRSVIG
      70      80      90      100     110     120

      130     140     150     160     170     180
g519-1.pep RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVFPQEIILRAMQAQITAERE
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVFPQEIILRAMQAQITAERE
      130     140     150     160     170     180

      190     200     210     220     230     240
g519-1.pep KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
      190     200     210     220     230     240

      250     260     270     280     290     300
g519-1.pep LVAEANAEAIRQIAAALQTGGADAVNLIKAEQYVAAFNNLAKESNTLIMPANVADIGSL
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      LVAEANAEAIRQIAAALQTGGADAVNLIKAEQYVAAFNNLAKESNTLIMPANVADIGSL
      250     260     270     280     290     300

      310
g519-1.pep ISAGMKIIDSSSKTAKX
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
m519-1      ISAGMKIIDSSSKTAKX
      310

```

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

```

a519-1.seq
1  ATGGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51  ATCCTTTGTT GTCATCCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGGTTTCCA TCGGCGGCTG ACGGCGGTTT TGAATATTTT GATTCCCTTT
151 ATCGAGCCGC TCGCTTACCG CCKTTCCGTT AAGAAATCCG CTTTACGAGT
201 ACCGAGCCAG GTCTGCATCA CGCGGACAAA TACCGAGCTG ACTGTGAGG
251 GTATCATCTA TTTCGAAGTA ACCGACCCCA AACTCGGCTC ATACGTTGCG
301 AGCAACTACA TTATGCGGAT TACCACGCTT GCCCAACGGA CGCTGCGTTC
351 CGTATCGGGG CGTATGGAAT TGGACAAAAC GTTTGAAGAA CGGACAGAAA
401 TCAACAGCAC CGTCTCTCC GCGCTCGATG AAGCGCGGG AGCTTGGGGT
451 GTGAAGTTT TCGGTTATGA GATTAAAGAC TTGGTTCCGC CGCAGAAAT
501 CTTTGCCTCA ATCGAGCGCG AATTACTCTG TGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAATCG AACAATCAA CTTGCCAGT
601 GGTGAGCGCG AAGCGGAAT CCAACAAAT CC GAAGCGGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAAATGCG AGAAATCGC CGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTGTGTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAAATT GCGCGCGCCT TCAAACCCAA GCGGTTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAA ATCGTCGCGC GTTCAACAA CTTGCCAAAG
851 AAAGCAATAC CTGATTATG CCGGCCAATG TTGCGGACAT CGGCGAGCTG
901 ATTTCTGCGG GTATGAAAT TATCGACAGC AGCAAAACCG CCAAAATA

```

- 76 -

This corresponds to the amino acid sequence &lt;SEQ ID 19; ORF 519-1.a&gt;:

```

a519-1.pep.
1  MEFFITLLAA VVVFQKSFV VIFQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAIRHSL KEIPLDVFSQ VCITRDNTQL TVDGIYFQV TDPKLASYGS
101 SNYIMATTQL AQTTILRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESG RKIEQINLAS
201 GQREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAENAEAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAPNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa
overlap

      10      20      30      40      50      60
a519-1.pep MEFFITLLAAVVVFQKSFVVIPQGEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL
          |||
m519-1      MEFFITLLVAVAVVFQKSFVVIPQGEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL
          |||
      10      20      30      40      50      60

      70      80      90     100     110     120
a519-1.pep KEIPLDVFSQVCITRDNTQLTVDGIYFQVTDPKLASYGSSNYIMATTQLAQTTILRSVIG
          |||
m519-1      KEIPLDVFSQVCITRDNTQLTVDGIYFQVTDPKLASYGSSNYIMATTQLAQTTILRSVIG
          |||
      70      80      90     100     110     120

      130     140     150     160     170     180
a519-1.pep RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVFPQEILRSMQAQITAERE
          |||
m519-1      RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVFPQEILRSMQAQITAERE
          |||
      130     140     150     160     170     180

      190     200     210     220     230     240
a519-1.pep KRARIAESEGRKIEQINLASGQREAEIQSQSEGEAQAAVNASNAEKIARINRAKGEAESLR
          |||
m519-1      KRARIAESEGRKIEQINLASGQREAEIQSQSEGEAQAAVNASNAEKIARINRAKGEAESLR
          |||
      190     200     210     220     230     240

      250     260     270     280     290     300
a519-1.pep LVAENAEAIRQIAAALQTQGGADAVNLKIAEQYVAAPNNLAKESNTLIMPANVADIGSL
          |||
m519-1      LVAENAEAIRQIAAALQTQGGADAVNLKIAEQYVAAPNNLAKESNTLIMPANVADIGSL
          |||
      250     260     270     280     290     300

      310
a519-1.pep ISAGMKIIDS SKTAKX
          |||
m519-1      ISAGMKIIDS SKTAKX
          |||
      310

```

576 and 576-1 gnm22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

```

m576.seq.. (partial)
1  ..ATGCAGCAGG CAAGCTATGC GATGGCGCTG GACATCGGAC GCTCCCTGAA
51  GCAATGAAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAG
101 CCATGCAGGC AGTGATATGAC GGCAGAGAAA TCAAAATGAC CGAAGAGCAG
151 GCTCAGGAGG TCATGATGAA ATTCTCTCAG GAACAACAGG CTAAGCCGT
201 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GCGAAGCCT

```

- 77 -

```

251 TTCTGAAAGA AAATGCCGCC AAGACGGCGG TGAAGCCAC TGCTCCGGC
301 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAACACAG CGACCAAAAG
351 CGACATCGTT ACCGTGGAAT ACGAGGCGCG CCTGATTGAC GTTACGGTAT
401 TGGACAGCAG CAAAGCCAAAC GCGGCGCGCG TCACCTTCCC TTTGAGCCAA
451 GTGATTCGGG GTTGGACCGA AGCGGTACAG CTCTCTGAAA AAGCGCGCGA
501 AGCAGCTTC TACATCCCGT CCAACCTTGC CTACCGCGGA CAGGGTGGCG
551 GGCACAAAT CGGTCCGAC GCACCTTGG TATTGATGT GAACCTGTCT
601 AAATCGCGG CACCCGAAA OCGCGCGCC AAGCAGCGG CTCAGTCTGA
651 CATCAAAAA GTAARTTA

```

This corresponds to the amino acid sequence <SEQ ID 21; ORF 576>:

```

m576.pep.. (partial)
1 ..MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
51 AEEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
101 LQYKTKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
151 VIPGWTEGVQ LKEGGEATF YIPSNLAYRE QGAGDKIGFN ATLVDVVKLV
201 KIGAFENAFK KQPAQVDIKK VN*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 22>:

```

g576.seq.. (partial)
1 ..atgggcgtgg acatcggcag ctccttgaaa caaatgaagg aacagggcgc
51 ggaatcgat ttgaaagtct ttaccgatgc catgcaggca gtgatgatgc
101 gcaaaagaaat caaatgacc gaagagcagg cccaggaagt gatgatgaaa
151 ttctctgcaag agcagcaggc taagccgcta gaaaaacaca agcgggatgc
201 gaaggccaac aaagaaaaag gcgaagcctt cctgaaggaa aatgccgccg
251 aagacggcgt gaagaccact gcttcgggtc tgacgtacaa aatcaccaaa
301 cagggtgaag gcaaacagcc gacaaaagac gacatcgtaa ccgtggaata
351 cgaaggccgc ctgattgacg gtaccgtatt cgacagcagc aaagccaacg
401 gcggcccgcc cacttcctct ttgagccaag tgattccggg ttggaccgaa
451 ggcgtacggc ttctgaaaga agcgccgcaa gccactgtct acatccggtc
501 caaccttggc taccgcgaac aggggtcggg cgaaaaaatc gggtccgaac
551 ccactttggt atttgacgtg aaactgggtc aaatcggcgc acccgaaac
601 gcgcccgcca agcagccgga tcaagtgcac atcaaaaaag taatttaa

```

This corresponds to the amino acid sequence <SEQ ID 23; ORF 576.ng>:

```

g576.pep.. (partial)
1 ..MGVDIGRSLK QMKEQGAIED LKVFTDAMQA VYDGKEIKMT EQQAEVMMK
51 FLQEQQAKAV EKHKADAKAN KEKGEAFLEK NAEEDGVKTT ASGLQYKITK
101 QGEGKQPTKD DIVTVEYEGR LIDGTVFDSS KANGGPATFP LSQVIPGWTE
151 GVRLLKEGGE ATFYIPSNLA YREQGAGEKI GPNATLVFDV KLVKIGAPEN
201 APAKQPDQVD IKKN*

```

Computer analysis of this amino acid sequence gave the following results:

Homology with a predicted ORF from *N. gonorrhoeae*

```

m576/g576 97.2% identity in 215 aa overlap
              10      20      30      40      50      60
m576.pep  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYDGKEIKMTEEQ QAEVMMKFLQ
g576      MGVDIGRSLKQMK EQGAEIDLKV FTEAMQAVYDGKEIKMTEEQ QAEVMMKFLQ
              10      20      30      40      50
m576.pep  EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASGLQYKITK QGEGKQPTKDDIV
g576      EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASGLQYKITK QGEGKQPTKDDIV
              60      70      80      90      100     110     120

```

- 78 -

```

              130      140      150      160      170      180
m576.pep      TVEYEGRLIDGTVFDSSKANGGPFVTFPLSQVIPGWTEGVLLKKEGGEATFYIPSNLAYRE
              |||
g576           TVEYEGRLIDGTVFDSSKANGGPFVTFPLSQVIPGWTEGVLLKKEGGEATFYIPSNLAYRE
              120      130      140      150      160      170

              190      200      210      220
m576.pep      QGAGDKIGFNATLVFDVCLKVIGAPENAPAKPQAVDIKKVNX
              |||
g576           QGAGDKIGFNATLVFDVCLKVIGAPENAPAKPQAVDIKKVNX
              180      190      200      210

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 24>:

```

a576.seq
1      ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51     ACTTTCGCGC TGCGCGCAAAA AACCAAGCCG CCCCAGCATCT GCATCCGAAC
101    ATCGCGCGCG TTCTTCCCGG CAGGGCGACA CCTCTTCGAT CGCGACGACG
151    ATCGACGACG CAACGCTATGC GATGGGGCTG GACATCGAAG GCTCCCTGAA
201    GCAATGCAAG GAACAGGCGCG CGGAATCGA TTTGAAGTC TTTACCGAAG
251    CCATCGAGCG ACTGTATCAG GCGAAGAAA TCAAATGAC CGAAGACGAG
301    GCTCAGGAAG TCATGATGAA ATTCTTCAG GAACAACAGG CTAAGCCGCT
351    AGAAAAACAC AAGCGCGACG CGAAGGCCAA TAAAGAAAA GCGCAAGCCT
401    TTCTGAAGA AATCGCGCGC AAAGCGCGCG TGAAGACAC TGCTTCGCGC
451    CTGCAATACA AATCACCATA ACAGGGCGAA GGCAACACG CGACCAAGA
501    CGACATCGTT ACCGTGGAAT ACGAAGCCG CCTGATTGAC GGTACGGTAT
551    TCGACAGCAG CAAGCGCAAC GCGGCGCGG TCACCTTCCC TTTGAGCCAA
601    GTGATTCTGG GTTGGACCGA AGGCGTACAG CTCTTGAAG AAGCGCGCGA
651    AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGSGTGGCG
701    GCGACAAAAT CGGCCGAAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
751    AAAATCGCGC CACCGAAAA CGCGCCGCC AAGCAGCGCG CTCAACTCGA
801    CATCAAAAAA GTAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 25; ORF 576.a>:

```

a576.pep
1      MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST
51     MQQASYAMGV DGRSLKQMK EQGAEIDLKV FTEAMQAVYD GREIKMTEEQ
101    AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVTITASG
151    LQYKITQGE GRQPTKDDIV TVEYEGRLID TVFDSSKAN GPFVTFPLSQ
201    VILGWTEGVQ LKFGGGEATF YIPSNLAYRE QGAGDKIGFN ATLVFDVCLKV
251    KIGAPENAPA KQPAQVDIKK VN*

m576/a576     ORFs 576 and 576.a showed a 99.5% identity in 222 aa overlap

              10      20      30
m576.pep      MQQASYAMGV DGRSLKQMK EQGAEIDLKV
              |||
a576           CGKKEAAPASASEPAASSAQGDTSSIGSTMQQASYAMGV DGRSLKQMK EQGAEIDLKV
              30      40      50      60      70      80
              40      50      60      70      80      90
m576.pep      FTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKHKADAKANKEKGEAFLENAA
              |||
a576           FTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKHKADAKANKEKGEAFLENAA
              90      100     110     120     130     140
              100     110     120     130     140     150
m576.pep      KDGVKTTASGLQYKITQGEKGQPTKDDIVTVEYEGRLIDGTVFDSSKANGGPFVTFPLSQ
              |||
a576           KDGVKTTASGLQYKITQGEKGQPTKDDIVTVEYEGRLIDGTVFDSSKANGGPFVTFPLSQ
              150     160     170     180     190     200

```



ms576.pep  
a576

160 170 180 190 200 210  
VIPGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGNATLVFDVKLVKIGAPENAPA  
|||  
VILGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGNATLVFDVKLVKIGAPENAPA  
210 220 230 240 250 260

ms576.pep  
a576

220  
KQPAQVDIKKVNX  
270  
KQPAQVDIKKVNX  
270

```

m576-1.seq
1  ATGAACACCA  TTTTCAAAT  CAGCGCACTG  ACCCTTTCCG  CCGCTTTGGC
5  ACTTTTCGCC  TGGCTGAAA  AAGAAGCGCG  CCGCGCATCT  GCATCGGAAC
101  CTGCGCGCGC  TCTTTCGGC  CAGGGCGACA  CCTTCTGAT  CGGACGACAG
151  ATGACGACGG  CAACTATG  CAGGATGATG  GACATCGSAC  GTTCCCTGAA
201  GCAATGAAG  GACAAGCGC  OGGAAATCGA  TTGAAAGCT  TTATCCGAAG
251  CCATGCGAGC  AGTGTATAG  GGCAAGAAAG  TCAAAAGTAC  CGAAGAGCGT
301  GCTGACGAG  TCA'TGATGA  ATTCTTCACG  GACAAACAG  TTAAGACCGT
351  AGAAAAGAAC  AAGGGCGGCG  CAAAGCGGCA  TAAAGAAAGA  GCGGACGCGT
401  T'TCTGAAGA  AAA'TCGCCG  AAGACGCGCG  TGAAGAACCA  TGCT'TCGCG
451  CTGCAATATA  AAA'TCACAA  CAGAGCGGCA  GGCAAAACAG  GACCAAAAGA
501  CGACATCGTT  ACCGTGGAA  ACAGGAGGCG  CCTGATGAC  GGTGACGTAT
551  TGCACGACG  CAAAGCCAA  GCGGGCCGCG  TCACCTTCCC  TTATGAGCSA
601  CTGATTCCCG  GTTGGACGAG  AGGCGTACAG  TCT'CTGAAG  AAGGCGCGAA
651  AGCCACATTT  TACATCCGAT  CCAACTTTCG  CTGACGOGAA  CAGGSGTGGT
701  CCCACAAAT  CGGTCCGAAT  GCGACTT'TG  TTAT'TGATG  GAACTGCTCT
751  AAAATCGCG  CACCAGAAAA  CCGCGCCOCC  AAGCAGCCGG  CTCAGTGA
801  CATCAAAAA  GTAATTAA

```

```
m576-1.pep
1  MNTIFKISAL TLSAALALSA CCKKEAAPAS ASEPAASSA QGDSSIGST
51  MQQASVAMVG DGRSLKQMK EGGAEIDLKV FTEAMAVDV GREIIMTEQ
101  AEQVNMFKQGE EQQAKAEVDE KADAKANEKK GEAFLKSNAN KGQVITFASG
151  LQYKTIKQGE GKQPTKDDIV TVEYGRILDI GVTVDSSKAN GGPVTFPLSG
201  VIPGTGTEGV LLKEGGATEV IYPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251  KIGAPENNA KPCAPCDIKK VN*
```

g576-1.seq

1	ATGACACCA	TTTTCAAA	CAGCGCACTG	ACCTTTTCGC	CCGCTTTGGC
5	ACTTTTCGCG	TGCGGCAAAA	AAGAGCGGCA	CCCGCATCTT	GGATCCGAC
101	CTGCGCGCGC	TTTTCGCGCG	CAGGCGCGCA	CCTCTTCAAT	CGGACGACG
151	ATCAGCAGAG	CAACTATGAT	AATGGCGGTG	GACATCGGAC	GCTTCCCTAA
201	ACCAATGARG	GACAGCGGAG	CGGAAATGGA	TTTGAAGATG	TTTACCGTAG
251	CCATCGAGCG	AGTGTATGAC	GCGAAGAAAC	TCAAAGATGC	CGAAGACGAG
301	GCCGAGGARG	TGATGATGAT	ATTTCTCGAG	GAGCAGCAGG	CTAAAGCCGT
351	AGAAAACAC	AGAGCGGATG	CGAAGCGGCA	CAAGAAGAAA	GGCGAAGCCT
401	TCTTGGAAGA	AAATGGCGCG	AAAGCAGCGG	TGAAGACCAT	TGCTTTCGGT
451	CTCGAGTACA	AAATCACCAA	CAGGGTGAAG	GGAAAGACCC	CACAAAGAAG
501	CGACATCGTT	ACCGTGGAA	CAGAGGCGCG	CTTGATGATG	GGTAGCGTAT
551	TGACAGCAGC	CAAGCGCGAC	GCGGCGCCGC	CAACCTTCCG	TTTACGCCAA
601	GTGATTCCGG	TTTGAGCGAG	AGGGCTACGG	TTCTCGAGAA	AAGCGGGGGA
651	ACCCACCTGT	TACATCCCGT	CCAACCTTGC	CTTACGGGAA	CACGCTGGGT
701	CCCAAAAATC	CGGTCGGAAC	GCGACCTTGG	TATTTCAGCG	GAACTGGTCT
751	AAATTCGGCG	CACCCGAAAC	CCGCAATGCG	AAGGACCGCG	ATCAAGTCGA

- 80 -

801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence &lt;SEQ ID 29; ORF 576-1.ng&gt;:

g576-1.pep

```

1  MNTIFKISAL TISAALALSA CGKKEAAPAS ASEPAASAA QGDTSSIGST
51  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQ
101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITQGE GKQPTKDDIV TVPYEGRLLD GTVFDSSKAN GGPATFPLSQ
201 VIPGWTEGVR LKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPDQVDIKK VN*

```

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

	10	20	30	40	50	60
g576-1.pep	MNTIFKISAL	TISAALALSA	CGKKEAAPAS	ASEPAASAA	QGDTSSIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TISAALALSA	CGKKEAAPAS	ASEPAASAA	QGDTSSIGST	MQQASYAMGV
	10	20	30	40	50	60
g576-1.pep	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEQ	AQEVMKFLQ	EQQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEQ	AQEVMKFLQ	EQQAKAVEKH
	70	80	90	100	110	120
g576-1.pep	KADAKANKEK	GEAFLENAA	KDGVKTTASG	LQYKITQGE	GKQPTKDDIV	TVPYEGRLLD
m576-1	KADAKANKEK	GEAFLENAA	KDGVKTTASG	LQYKITQGE	GKQPTKDDIV	TVPYEGRLLD
	130	140	150	160	170	180
g576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
	190	200	210	220	230	240
g576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
	250	260	270			
g576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
	250	260	270			

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq

```

1  ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCGG CCGCTTTGGC
51  ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCGCGCGC TTCTTCGCGC CAGGGCGACA CCTCTTCGAT CGGCAGCACG
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAATGGAAG GAACAGGCGC CGGAAAACGA TTTGAAGTCT TTTACCGAAG
251 CATGCAGCGC AGTGTATGAC GGCAGAGAAA TCAGAAATGAC CGAGAGCAGC
301 GCTCAGCAGG TCATGATGAA GTTCTCTCAG GAACACACAG TAAAGCCGCT
351 AGAAAAACAC AAGCGCGAAG CGAAGGCCAA TAAAGAAAAA GCGGAAGCCT
401 TTCTGAAGAA AATTCGCGCC AAGACGCGCG TGAAGACCAC TGCTTCGGCG
451 CTGCAATACA AATTCACCAA ACAGGCGGAA GCGAACACAG CGACCAAGAA
501 CGACATCGTT ACCGTGGAAT ACGAAGCCGC CCTGATTGAC GGTACGTTAT
551 TCGACAGCAG CAAAGCCAAAC GGCGCCCGCG TCACCTTCCC TTTGAGCCAA
601 GTGATTCTGG TGTGGACCGA AGGCGTACAG CTTCTGAAG AAGGCGGCGA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGTCGCGC
701 GGCACAAAAT CGGCCCGAAC GCACACTTGG TATTTGATGT GAAACTGTCT

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- 81 -

751 AAAATCGGCG CACCCGAAAA CGGCGCCGCC AAGCAGCCGG CTCAAGTCGA  
801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep  
1 MNTIFKISAL TISAALISA CGKKEAAPAS ASEPAASSA QGDTSSIGST  
51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ  
101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG  
151 LQYKITQKGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ  
201 VILGWTEGVQ LKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVDVFKLV  
251 KIGAPENAPA KQPAQVDIKK VN\*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

	10	20	30	40	50	60
a576-1.pep	MNTIFKISAL	TISAALISA	CGKKEAAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TISAALISA	CGKKEAAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV

	70	80	90	100	110	120
a576-1.pep	DIGRSLKQMK	EQGAEIDLK	VTEAMQAVY	DGKEIKMTEE	QAEVMMKFL	EQQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLK	VTEAMQAVY	DGKEIKMTEE	QAEVMMKFL	EQQAKAVEKH

	130	140	150	160	170	180
a576-1.pep	KADAKANKEK	GEAFLENAA	KDGVKTTAS	GLQYKITQKG	EGKQPTKDDI	VTEYEGRLID
m576-1	KADAKANKEK	GEAFLENAA	KDGVKTTAS	GLQYKITQKG	EGKQPTKDDI	VTEYEGRLID

	190	200	210	220	230	240
a576-1.pep	GTVFDSSKAN	GGPVTFPLS	QVILGWTEG	VQLKEGGEAT	FYIPSNLAYR	EQGAGDKIGPN
m576-1	GTVFDSSKAN	GGPVTFPLS	QVILGWTEG	VQLKEGGEAT	FYIPSNLAYR	EQGAGDKIGPN

	250	260	270
a576-1.pep	ATLVDFVKLV	KIGAPENAPA	KQPAQVDIKK
m576-1	ATLVDFVKLV	KIGAPENAPA	KQPAQVDIKK

919 and 919-2 gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq  
1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT  
51 CCTGCGCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA  
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCGACC  
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTG TATACCGTTG TACCGCACCT  
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTG GCCAAAAGC CTGCAATCCT  
251 TCCGCTCTGG CTGCGCCAAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG  
301 TGCGCCCAAG CCTTTCAAAC CCCCGTCCAT TCCTTTCAGG CAAAACAGTT  
351 TTTTGAAACG TATTTCACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG

- 82 -

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401 CCGGTACGGT TACCGGCTAT TAGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCGGATTAC GGTATTCGG ACGATTTTAT
501 CTCGCTCCCC CTGCTGCGG GTTTGGGGAG CGGAAAAGCC CTGTGCGGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGCGGCACAA
601 CATACCGCCG ACCTCTCCcG ATTCCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCGCGTTCT CCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GAGCGAAGG CCCGATACT CGGTACGGC
751 GAAGACCTCT TCGAACTTTT TTTTATGCAC ATCCAAGCT CGGCGCTCT
801 GAAAACCCCG TCGCGCAAAT ACATCCGATC CGCTATGCC GACAAAAAG
851 AACATCCyTA GCTTCCATC GGACGTATA TGCGGATAA GGGCTACCTC
901 AAATCGGAC AAACCTCCAT GCAGGCGATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCAAG TTTTGGGTCA AAACCCGAG TATATCTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCTCTGCG CGCACTGGGC
1051 ACGCGCGTGA TGGGGGAATA TGCGCGGCGA GTCGACGGC ACTACATTAC
1101 CTGGGTGCG CCCTTATTGT TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCTCAACCG CCTGATTATG GCGCAGGATA CCGCGAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GCGCGCAAG CCGCGGAAT
1251 TGCCGCGAAA CAGAAAAACA CGGGATATGT CTGGCAGCTC TACCCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919.pap

```

1 MKKYLFRAL YQIAAAILAA CQSKSIQTFF QPDTSVINGP DRPVGIPDPA
51 GTTVGGGAV YTVVPHLSL HWAQDFAKS LQSFRLGCAN LKNRQGNQDV
101 CAQAFQTFVH SFQAKQFFER YFTPMQVAGN GSLAGTVTGY YEPVLKGGDR
151 RTAQRFPFIY GIPDFIIVP LPAGLRSGKA LVRIRGTGNK SGTIDMTGGT
201 HTADLSRFFI TARTTAIKGR FEGRFLPYH TRNQINGAL DGKAPILGYA
251 EDPELVFMH IQSGSRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQSI KSYMQRNPQR LAELVGNQNS YIFRELAGS SNDGFWALG
351 TPLMGYAGA VDRHYITLGA PLFVAIAHPV TRKALNKLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTGYWQL LPNGMKPEYR P*

```

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

a919-2.seq

```

1 ATGAAAAAAT ACCTATTCCG CGCGCCCTG TACGGCATCG CGCGCGCCAT
51 CCTCGCGGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCGCTCAT CAACGCCCGG GACCGGCCGG TCGCATCCC CGACCCGCGC
151 GGAACGACGG TCGCGCGCGG CGGGCGCGTC TATACCGTTG TACCGCACCT
201 GPCCCTGCCC CACTGCGGCG CGCAGGATTT CGCAAAAAGC CTGCAATCCT
251 TCGGCTCGGG CTGCGCCAAT TTGAAAAACC GCGAAGGCTG CGAGGATGTG
301 TGCGGCCAAG CCTTTCAAAC CCCCTGCCAT TCCTTTGAGG CAATAACGTT
351 TTTTGAACGC TATTTCAAGC CGTGCGAGGT TCGAGGCAAC GGAAGCCTTG
401 CGGTCACGGT TACCGGCTAT TAGCAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCGGATTAC GGTATTCGGC ACGATTTTAT
501 CTCGCTGCCC CTGCTGCGG GTTTGGGGAG CGGAAAAGCC CTGTGCGSCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGCGGCACAA
601 CATACCGCGC ACCTCTCCcG ATTCCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCGCGTTCT CCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GAGCGAAGG CCCGATACT CGGTACGGC
751 GAAGACCTCT TCGAACTTTT TTTTATGCAC ATCCAAGCT CGGCGCTCT
801 GAAAACCCCG TCGCGCAAAT ACATCCGATC CGCTATGCC GACAAAAAG
851 AACATCCCTA GCTTCCATC GGACGTATA TGCGGATAA GGGCTACCTC
901 AAATCGGAC AAACCTCCAT GCAGGCGATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCAAG TTTTGGGTCA AAACCCGAG TATATCTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCTCTGCG CGCACTGGGC
1051 ACGCGCGTGA TGGGGGAATA TGCGCGGCGA GTCGACGGC ACTACATTAC
1101 CTGGGTGCG CCCTTATTGT TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCTCAACCG CCTGATTATG GCGCAGGATA CCGCGAGCGC GATTAAAGGC

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- 83 -

1201 GCGGTGCGCG TGGATTATT TTGGGGATAC GGCACGAAG CGGCGAACT  
 1251 TGCCGCGAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAAACG  
 1301 GTATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2 . pep

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINP DRPVGIPDPA  
 51 GTTVAGGGAV YTVVPHLSLP HWAADQFAKS LQSFRLGCAN LKNRQGWQDV  
 101 CAQAFQTPVH SFQAKOFFER YTPFWQVAGN GSLAGTVTGY YEPVLKIDDR  
 151 RTAQAARFPIY GIPDDFISVP LPAGLRSGKA LVRIQTGKN SGTIDNAGGT  
 201 HTADLSRFFPI TARITAIKGR FEGSRFLPYH TRNQINGGAL DGKAPILGYA  
 251 EDPVLELFMH IQSGSRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL  
 301 KLGQTSMQSI KSYMRQNPR LAELVGNQNS YIFRELKAGS SNGSPVAGL  
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG  
 401 AVRVDYFWGY GDEAGELAGK QKTTGYWQL LPNGMKPEYR P\*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919 . seq

1 ATGAAAAAAC ACCTGCTCCG CTCGCCCTG TAGCGcatCG CCGCGcctAT  
 51 CctcgCGGCC TGCCAAAGca gGAGCATCCA AACCTTTCCG CAACCCGACA  
 101 CATCCGTTCAT CAACGCCCGG GACCCGCCGG CCGCATCCCC CGACCCCGCC  
 151 GGAACGACAG TTGCGGCGCG CGGGGCCGTC TATACGTTTG TCCCGCACCT  
 201 GTCCATGCCCC CACTGGGCGG CGCaggATT TTGCCAAAGCG CTGCAATCCT  
 251 TCCGCTCCG CTGCGCCAAT TTGAAAAACC GCCAAGCGTG GCAAGATGTG  
 301 TGCGGCCAAG CCTTTCAAAC CCCCCTGCAT TCCTTTTCAAG CAAAGcggTT  
 351 TTTTGAAACG TATTTCAAGC cgtGGCaggT tgcaggcaAC GGAAGcCTTG  
 401 CaggtacggT TACCGGCTAT TACGAAACCGG TGCTGAAAGG GACCGGACGG  
 451 CGGACGGAAC GGGCCCGCTT CCGCATTTAC GGTATTTCCG ACGATTTTAT  
 501 CTCGTCCTCG CTGCTTCGCG GTTTGCGGGG CGGAAAAAAC CTTGTCCGCA  
 551 TCAGGACGac gggGAAAAAC AGCGGCACGA TCGACAAATCG CCGCGGACCG  
 601 CATACCGCGG ACCTCTCCCG ATTCCCATC ACCGCGCGCA CAACGGcaat  
 651 caaaGGCAGG TTTGAaggAA GCGCGTTCCT CCCTTACCAC ACGCGCAACC  
 701 AAATcaacCG CGGCGcgcTT GAOGGCAAG cccCATCCT CggttaagcC  
 751 GAgaccCcg tccaactTT TTTCATGCAT AtccaaggCT CGGGCCCGCT  
 801 GAAAAACCCG tccggcaaat acatCCGcAT oggaTaagcc gacAAAAACG  
 851 AACAtccgTa tgtttccatc ggACGctaTA TGGCGGACAA AGGCTACCTC  
 901 AAGctcgggc agACCTCGAT GCAGGgcatc aaagcCTATA TGCGGCAAAA  
 951 TCGGCAACGC CTGCGCGAAG TTTTGGGTGA AAACCCGAGC TATATCTTTT  
 1001 TCGCGAGCT TCGCGGAAGC GGCAATGAGG GCCCGTGG GCGACTGGCG  
 1051 ACGCCTATGA TGGGGGAATA CGCCCGCGCA ATCGACCGCG ACTACATTAC  
 1101 CTTGGGCGCG CCGTTATTGT TCGCCACGCG CCATCCGTT ACCCGCAAG  
 1151 CCTCAACCG CCGTATTATG GCGCAGGATA CAGGACGCG GATCAAAGGC  
 1201 GCGGTGCGCG TGATTTATT TTGGGTATC GGGCAGCAAG CCGCGAACT  
 1251 TGCCGCGAAA CAGAAAACCA CGGGATACGT CTGGCAGCTC CTGCCAAACG  
 1301 GCATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919 . pep

1 MKKHLLRSAL YGIAAAILAA CQSKSIQTFP QPDTSVINP DRPAGIPDPA  
 51 GTTVAGGGAV YTVVPHLSLP HWAADQFAKS LQSFRLGCAN LKNRQGWQDV  
 101 CAQAFQTPVH SFQAKOFFER YTPFWQVAGN GSLAGTVTGY YEPVLKIDDR  
 151 RTERARFPIY GIPDDFISVP LPAGLRSGKN LVRIQTGKN SGTIDNAGGT  
 201 HTADLSRFFPI TARITAIKGR FEGSRFLPYH TRNQINGGAL DGKAPILGYA  
 251 EDPVLELFMH IQSGSRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL  
 301 KLGQTSMQSI KAYMRQNPR LAELVGNQNS YIFRELKAGS GNEGSPVAGL  
 351 TPLMGEYAGA IDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG  
 401 AVRVDYFWGY GDEAGELAGK QKTTGYWQL LPNGMKPEYR P\*



- 85 -

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1  ATGAAAAAAT  ACCTATTCGG  CGCCGCCCTG  TGCGCATCG  CGCGCCCAT
51  CCTCGCCGCC  TGCCAAAGCA  AGAGCATCCA  AACCTTTCCG  CAACCCGACA
101 CATCGCTCAT  CAACGGCCCG  GACCGGCCGG  TCGGCATCCC  CGACCCCGCC
151 GGAACGACGG  TCGCGCGCGG  CGGGCCGCTT  TATACGCTTG  TGCCGCACTT
201 GTCCCTGCCC  CACTGGGCGG  CGCAGGATTT  GCCCAAAGAC  CTGCAATCCT
251 TCGCCTCGG  CTGGCCCAAT  TTGAAAAACC  GCCAAGCGTG  CGAGGATGTG
301 TGCGCCCAAG  CCTTTCAAC  CCCCTCCAT  TCCGTCAGG  CAAAACGATT
351 TTTTGAACGC  TATTTCAAGC  CTTGCGAGGT  TSCAGCAAC  GGAAGCGCTG
401 CGGTACGGT  TAATCGCTAT  TGCTAAGCGG  TGCTAAGCGG  CGACGACAGG
451 CGGACGGCAC  AAGCCGCTTT  CCCGATTATC  GGTATTCCCG  ACGATTTTAT
501 CTCCTCCCC  CTGCTCGCG  GTTTCGGAG  CGGAAAAGCC  CTTGTCGCA
551 TCAGGCAGAC  GGGAAAAAAC  AGCGGCACAA  TCACAATAC  CGCGGCACA
601 CATACCGCG  ACCTCTCCCA  ATTCGCCATC  ACTGCGCGCA  CAACGGCAAT
651 CAAAGGCAGG  TTTGAAGGAA  CGCGCTTCCT  CCCCTACCAC  ACGCGCAACC
701 AAATCAACGG  CGGCGCGCTT  GACGGCAAG  CCCGATACT  CGGTTACGCC
751 GAAGACCCCG  TCGAATCTTT  TTTTATGAC  ATCCAAGGCT  CGGGCCGCTT
801 GAAAACCCCG  TCCGGCAAT  ACATCCGAT  CGGCTATGCC  GACAAAAACG
851 AACATCCCTA  CGTTTCCATC  GGACGCTATA  TGGCGGACAA  AGGCTACCTC
901 AAGCTCGGGC  AGACCTCGAT  GCAGGGCATC  AAAGCCTATA  TGCAGCAAAA
951 CCCGCAACGC  CTCGCCAAG  TTTTGGGGCA  AAACCCGAGC  TATATCTTTT
1001 TCCGAGAGCT  TACCGGAAG  AGCAATGAC  GCCTGTGCG  GCGACTGGCG
1051 ACGCGCTGTA  TGGCGGAGTA  CGCCGGCGCA  GTCGACCGCG  ACTACATTAC
1101 CTTGGGCGCG  CCCTTATTGT  TCGCCACGCG  CCATCCGTT  ACCCGCAAG
1151 CCCTCAACCG  CCGTATTATG  GCGCAGGATA  CCGCGACGCG  GATTAAAGGC
1201 GCGGTGCGCG  TGGATTATTT  TTGGGGATAC  GCGCAGGAAG  CCGGCCAATC
1251 TGCCTGGCAA  CAGAAAACCA  CGGGATATGT  CTGGCAGCTT  CTGCCAACG
1301 GTATGAAGCC  GAGAAACCG  CGGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

```

a919.pep
1  MKKYLFRAL  CGIAAAILAA  CQSKSIQTFF  QPDTSVINGP  DRPVGIPDPA
51  GTTVGGGGA  YTVVPHLSLP  HWAAQDFAKS  LQSFRLGCAN  LKNRQGWQDV
101 CAQAFQTPVH  SVQAKQFFER  YFTPWQVAGN  GSLAGTVTGY  YEPVLKGDDR
151 RTAQRFFPIY  GIPDDFISVP  LPAGLRSGKA  LVRIQTGKN  SGTIDNTGGT
201 HTADLSQFPI  TARTTAIKGR  FEGSRFLPYH  TRNQINGGAL  DGKAPILGYA
251 EDPVELFFMH  IQGSGRLKTP  SGKYIRIGYA  DKNEHPYVSI  GRYMADKGYL
301 KLGQTSMQGI  KAYMQNQNPQ  LAEVLGNQPS  YIFPRELTGS  SNDGPVGA LG
351 TPLMGEYAGA  VDRHYITLGA  PLFVTAHPV  TRKALNRLIM  AQDTGSAIKG
401 AVRVDYFWGY  GDEAGELAGK  QKTTGYWQL  LPNGMKPEYR  P*

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m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

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m919.pep      10      20      30      40      50      60
MKKYLFRALYGLIAAAILAACQSKSIQTFFQPDTSVINGPDRPVGIPDPAGTTVGGGGA
|||||
a919          10      20      30      40      50      60
MKKYLFRALCGIAAAILAACQSKSIQTFFQPDTSVINGPDRPVGIPDPAGTTVGGGGA
|||||

m919.pep      70      80      90      100     110     120
YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSPQAKQFFER
|||||
a919          70      80      90      100     110     120
YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSPQAKQFFER
|||||

m919.pep      130     140     150     160     170     180
YFTPWQVAGNSLAGTVTGYEYEPVLKGDDRRTAQARFPIYIGIPDDFTSPLPAGLRSGKA
|||||
a919          130     140     150     160     170     180
YFTPWQVAGNSLAGTVTGYEYEPVLKGDDRRTAQARFPIYIGIPDDFTSPLPAGLRSGKA
|||||

m919.pep      190     200     210     220     230     240
LVRIQTGKNSGTIDNTGGTHTADLSRFTTARTTAIKGRFEGSRFLPYHTRNQINGGAL
|||||
a919          190     200     210     220     230     240
LVRIQTGKNSGTIDNTGGTHTADLSRFTTARTTAIKGRFEGSRFLPYHTRNQINGGAL
|||||

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- 86 -

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a919      LVRIRQQTGKNSGTIDNTGGTHTADLSQFFITARTTAIKGRFEGSRFLPYHTRNQINGGAL
           190      200      210      220      230      240

           250      260      270      280      290      300
m919.pep  DGKAPILGYAEDFVELFFMHIOGSGRLKTFSGKYIRIGYADKNEHFVVSIGRYMADKGYL
           |||
a919      DGKAPILGYAEDFVELFFMHIOGSGRLKTFSGKYIRIGYADKNEHFVVSIGRYMADKGYL
           250      260      270      280      290      300

           310      320      330      340      350      360
m919.pep  KLQGTSMOGIKSYMRFONFORLAELVIGONPSYIFFRELAGSSNDGVPVGLGTPLMGEYAGA
           |||
a919      KLQGTSMOGIKAYMOONFORLAELVIGONPSYIFFRELTGSSNDGVPVGLGTPLMGEYAGA
           310      320      330      340      350      360

           370      380      390      400      410      420
m919.pep  VDRHYITLGAPLFVATAHFVTRKALNRLIMAQDTGSAIKGAVRVDFWVGDEAGELAGK
           |||
a919      VDRHYITLGAPLFVATAHFVTRKALNRLIMAQDTGSAIKGAVRVDFWVGDEAGELAGK
           370      380      390      400      410      420

           430      440
m919.pep  OKTTGYVWOLLFNGMKPEYREX
           |||
a919      OKTTGYVWOLLFNGMKPEYREX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGGGATGCC GTACTGATAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCAGCG CTTTAGCCCC TACCCCGGCA GGTTACGCCG CCAATTGCTG
151 GATTTCGAGG ACACAGGCGC AGACGAACTG CACCGCAGCA GGATTTGTCT
201 GCAAGAACTC AGCCGCTTAT ATGCGCAAAAC GC CGCGCGAA CTGCTGTGCA
251 GTCAAAACCT GCACCGCTCC GACATTACCG CCCTCGCGCTG CCACGGGCAA
301 ACCGTCGAC AGCGCGCGGA ACACGGTTAC AGCATACAGC TTGCGGATTT
351 GCGCGTCTGT GCGGxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
401 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
451 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
501 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
551 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
601 xxxxxxCAGC TTCCTTACGA CAAAAACGGT GCAAAAGTCGG CACAAGGCAA
651 CATATTGCCG CAATGCTCG ACAGGCTGCT CGCCACCGCG TATTTCGCAC
701 AACGCCACCC TAAAGCACG GGGCGCGAAC TGTTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGCGCG CGAAACCGGA TACGACGTAT TCGGGACGCT
801 TTCCCGTTTT AC CGCGCAAA CCGTTTGGCA CGCGCTCTCA CACGCAAGCG
851 CAGATTGCCG TCAAAATGTAC ATTTGGCGAG GCGGCATCCG CAATCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCTGTACAGC
951 CACCGCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCGGnATTGT
1001 CGTGGTTGSC GCGGCTGTGG ATTAATGCGA TTCCCGGTAG TCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG AnCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence &lt;SEQ ID 41; ORF 121&gt;:

m121.pep

```

1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHPTP YFGRLLRRLL
51  DLQDTGADEL HRSRLISQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGQ

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- 87 -

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101 TVRHAPHEGY SIQLADLPLI Axxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
151 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
201 xxQLPYDKNG AKSAQGNILP QLLDRLLAHP YFAQRHFKST GRELFALINWL
251 ETYLDGGENR YDVLRLSLRF TAQTVCDVAV HAAADARQMY ICDGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAACAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGGACGG
51 GCGGATGCC GTGCTGTAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCAGCG CTTTACCCCC TACCCTGACC GGTTCGCGCG CAATTTGCTG
151 GATTTCGAGG ACACAGGCAC AGACGAAGCT CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCGCGCTGT ACGCACAAAC CGCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCTCGCTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCGAC ACSCGCGGGA ACACGGTtac AGCATAACAG TTGCCGATTT
351 GCGCTGCTGT GCGGAACCTG cgcggatttT TACCGTCggc gacttccGCA
401 GCGCGACCTT TGCTCGCGCG GcaacagGTG CCGCGCTCTT CCGCGCTTCT
451 CACGAGACCC TCTTCGCGGA TGACAGGGA ACACGCTGTG TACTGAACAT
501 CCGCGGATTT GCCAACATCA GCGTACTICC CCGCGCGGCA CCGCGCTTCT
551 GCTTCGACAC AGGCGCGGCG AATATGCTGA TGGAcgctg gacgcaggca
601 cactGGcagc TGCTTACGA CAAAAacggt qcAAAgcgg ccaAAGCNA
651 catatTGCcg cAACTGCTCG gcaggtGCT CGCCcaecG TATTTCTCAC
701 AACCCcaccc aaAAGCACG GgCGGGAac TgtttgcccT AAattgctc
751 gaaacctAcc ttgacggcg cgaaaccca taagcagtat tgcggacgt
801 ttcccgattc accgcgcaaA ccgTttggga cgccgttcca CACGACGCGG
851 CAGATGCCCG TCAATGTAC ATTTCGGGCG GCGGCATCCG CAATCTGTT
901 TTAATGGCGG ATTTGGCAGA ATTTTCGGCG ACACGCGTTT CCCTGCACAG
951 CACCGCGGAA CTGAACCTCG ATCCTCAATG GGTGGAGCGG gccgCATTtg
1001 cgtggttggC GGCCTGTGG ATTAACCGCA TTCGCGTAG TCGCACAAA
1051 GCGACGCGCG CATCCAAACC GTGTATTCTG GCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMGDADA VLVRMDGGKW LGAEGHAFPT YPDRLRKLL
51 DLQDTGTDEL HRSRMLSQEL SRLYAQTAAE LLCQNLAFC DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLI AELTRIFTYD DFRSRDLAAG GQGAPIVPAP
151 HEALFRDDRE TRVVLNIGI ANISVLPGA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLGRLLAHP YFSQPHFKST GRELFALINWL
251 ETYLDGGENR YDVLRLSLRF TAQTVCDVAV HAAADARQMY ICDGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFAWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPYFGRLLRQLLDLQDTGDAEL					
g121	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPYFGRLLRQLLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRLYAQTAAELLSQNLA <sup>PS</sup> DITALGCHGQTVRHAPHEHGYSIQLADLPLI					
g121	HRSRILSQELSRLYAQTAAELLSQNLA <sup>PC</sup> DITALGCHGQTVRHAPHEHGYSIQLADLPLI					
	70	80	90	100	110	120
	130	140	150	160	170	180

- 88 -

```

m121.pep  AXXXXXXXXXXXXXXXXXXXXXKXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
           | : : : : :
g121      AELTRIFTVGDFRSRDLAAGQGQAPLVPAFHEALFRDRETRVLNIGGIANISVLPPGA
           130 140 150 160 170 180
           190 200 210 220 230 240
m121.pep  XXXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST
           : : : : :
g121      PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLGRLLAHPYFSQPHPKST
           190 200 210 220 230 240
           250 260 270 280 290 300
m121.pep  GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDVSHAAADARQMYICGGIRNPV
           | : : : : :
g121      GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDVSHAAADARQMYICGGIRNPV
           250 260 270 280 290 300
           310 320 330 340 350 360
m121.pep  LMADLAECFGTRVSLHSTADLNLDPOQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
           | : : : : :
g121      LMADLAECFGTRVSLHSTADLNLDPOQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
           310 320 330 340 350 360

m121.pep  XAGYYYY
           | : : : :
g121      GAGYYYY

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 44>:

```

a121.seq
1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGGATGCC GTACTGATAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCAGCG CTTTACCCCC TACCCCGGCA GGTTACGCCG CAAATTGCTG
151 GATTTCAGAG ACACAGGCGC GGACGAACTG CACCGCAGCA GATGTGTGTC
201 GCAAGAACTC AGCGCGCTGT ACGCGCAAAC CGCCCGCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCGCTTC GACATTACCG CCTCTGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCGCGA ACACAGTAC AGGTACAGC TTGCGGATTT
351 CGCGTGTCTG GCGGAACGGA CTCAGATTTT TACGTCGGC GACTTCGCGA
401 CGCGGACCTT TCGCGCGCGC GGACAGGGCG CGCGCTCGT CCGCGCTTT
451 CACGAAGCCC TGTTCGCGGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGCGGGGATT GCCAACATCA GCGTACTCCC CCGCGACGCA CCGGCTCTCG
551 GCTTCGACAC AGGACCGGCG AATATGCTGA TGGACGGTGT GATGCAGGCA
601 CACTGGCAGC TTCTTACGTA CAAAACCGT GCAAAGCGGG CACAAGGCAA
651 CATATTGCGG CAATGCTGCG ACAGGCTGCT CGCCACCGCG TATTTCGCAC
701 AACCCACACC TAAAGCGACG GGGCGGGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAACCGGA TACGAGCTAT TGGGAGAGCT
801 TTCCCGATCC ACCCGCGAAA CCGTTTTCGA CCGCGCTCTA CACGACAGCG
851 CAGATGCGCG TCAATGTGAC ATTGTTGCGG CGCGCATCGG CATTCCTGTT
901 TTAATGGCGG ACTTGGCAGA ATGTTTTCGAC ACACGCGTTT CCGTGCACAG
951 CACGCGCGAA CTGAACCTCG ATCCGCAATG GGTAGAGGCC GCGCGGTTGG
1001 CATGATGGCG GCGGTGTTGG GTCAACGCGA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG GCGCGGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 45; ORF 121.a>:

```

a121.pep
1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAECHAFTP YPGRLRRKLL
51  DLQDTGADEL HRSRLMSQEL SRLYAQTAAE LLCSQNLAFS DITALGCHGQ
101 TVRHAPHSY SVOLADLPIL ERTQIFTVG DFRSRDLAAG GGSFVYFAF
151 HEALFRDRE TRAVLNIGGT ANTSLVPPDA PAFGFDTPG NMLDANWQA
201 HWQLPYDRNG AKAAQGNILP LLDRLLAHP YFAQPHPKST GRELFALNW
251 ETYLDGGENR YDVLRTLSRF TACTVFDVAV HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAWMAACW VNRIPGSPHK

```

- 89 -

351 ATGASKPCIL GAGYYY\*

m121/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

	10	20	30	40	50	60
m121.pep	METQLYTGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFPPYGRLLRQLLDLQDTGADL					
a121	METQLYTGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFPPYGRLLRQLLDLQDTGADL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRILYAQTAAELLCSQLNAPSDITALGCHGQTVRHAFEHGYSIQLADLPL					
a121	HRSRILSQELSRILYAQTAAELLCSQLNAPSDITALGCHGQTVRHAFEHGYSIQLADLPL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121.pep	AXXX					
a121	AERTQIFTVGDFSRDLAAGGQGAFLVPAFHEALFRDRETRAVLNIGGIANISVLPDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121.pep	XXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPOLLRLLAHPFYFAQRHPKST					
a121	PAFGFDTPGPNMLMDAWMQAHNLQPYDKNGAKAAQGNILPOLLRLLAHPFYFAQRHPKST					
	190	200	210	220	230	240
	250	260	270	280	290	300
m121.pep	GRELFALNWLETYLDGGENRYDVLRTLRSRTAQTVDVAVSHAAADARQMYICDGGIRNPV					
a121	GRELFALNWLETYLDGGENRYDVLRTLRSRTAQTVDVAVSHAAADARQMYICDGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121.pep	LMADLAECFQTRVSLHSTADLNLDPQWVEAAAFWLAACWINRIFGSPHKATGASKPCIL					
a121	LMADLAECFQTRVSLHSTAEALNLDPQWVEAAAFWMAACWVNRIFGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121.pep	XAGYYYY					
a121	GAGYYYY					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 46>:

m121-1.seq

1	ATGGAACAC	AGCTTTACAT	CGGCATCATG	TCGGGAACCA	GCATGACGG
51	GGCGGATGCC	GTAATGATAC	GGATGAGCGG	CGGCAATGG	CTGGGCGCGG
101	AAGGGCACGC	CTTTACCCCG	TACCCCGGCA	GGTTACGCCG	CGAATTGCTG
151	GATTTCGAGG	ACACAGGCGC	AGACGAAGTC	CACCGCAGCA	GGATTTTGTC
201	GCAAGAACTC	AGCCGCCCTAT	ATGCGCAAAC	CGCCGCCGAA	CTGCTGTGCA
251	GTCRAAACTC	CGCACCGTCC	GACATTAACC	CCCTCGCGTG	CCACGGGCAA
301	ACCGTCCGAC	ACGGCGCGGA	ACACGGTTAC	AGCATACAGC	ITGCGGATTG
351	CGCGCTGCTG	GCGGAACGGA	CGCGGATTTT	TACCGTCGGC	GACTTCGGCA
401	GCGCGACACT	TGCGGCGCGC	GGACAAGGCG	CGCCACTCGT	CCCGGCTTTT
451	CACGAAGCCC	TGTTCCGCGA	CAACAGGGAA	ACACGCGCGG	TACTGAACAT
501	CGCGGGGATT	GCCAAATCA	GGTACTCCCG	CCCGACAGCA	CCCGGCTTCG
551	GCTTCGACAC	AGGGCGGGGC	AATATGCTGA	TGGACGCGTG	GACGACAGGA
601	CACTGGCAGC	TTCTTACGA	CAAAAACGGT	GCAAAGGCGG	CACAGGCAAA
651	CATATTCGCG	CAACTGCTCG	ACAGGCTGCT	CGCCACCGCG	TATTTGCGAC
701	AAACCCACCC	TAAAGCACG	GGGCGCGAAC	TGTTTGCCCT	AAATTGGCTC
751	GAAACCTACC	TTGACGCGG	CGAAAACCGA	TACGACGTAT	TGCGGACGCT

- 90 -

```

801 TTCCCGTTTT ACCGCGCAAA CCGTTTGGCA CGCGTCTCA CACGCAGCGG
851 CAGATGCGCG TCAAAATGAC ATTTGCGGCG GCGCATCCG CAATCTGTT
901 TTAATGCGCG ATTTGCGAGA ATGTTTCCGG ACACGCGTTT CCCTGCACAG
951 CACCGCGGAC CTGAACCTCG ATCCGCAATG GGTGAAGCC GCGNATTGT
1001 CGTGGTGGCG GCGCTGTGG ATTAATCGCA TTCCCGGTAG TCGGCACAAA
1051 GCAACGCGCG CATCCAAACC GTGTATTCTG ANCGCGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

```

m121-1.pep
1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGRLRRQLL
51  DLQDTGADEL HRSRILSOEL SRLYAQTAAE LLCSONLAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AERTRIFTVG DFRSRDLAAG GQGAFLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTPGPG NMLMDAWTQA
201 HWQLFYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICGGGIRNPV
301 IMADLAECFPG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYY

m121-1/g121 ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa
overlap

      10      20      30      40      50      60
m121-1.pep METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEGHAFTPYPGRLRRQLLQDTGADEL
g121        METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEGHAFTPYDRLRRKLLDQDTGTDEL
      10      20      30      40      50      60

      70      80      90     100     110     120
m121-1.pep HRSRILSQELSRSLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL
g121        HRSRILSQELSRSLYAQTAAELLCSONLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL
      70      80      90     100     110     120

      130     140     150     160     170     180
m121-1.pep AERTRIFTVGDFRSRDLAAGGQGAFLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA
g121        AELTRIFTVGDFRSRDLAAGGQGAFLVPAFHEALFRDRETRVNLNIGGIANISVLPPGA
      130     140     150     160     170     180

      190     200     210     220     230     240
m121-1.pep PAFGFDTPGPGNMLMDAWTQAHWQLPYDRNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST
g121        PAFGFDTPGPGNMLMDAWTQAHWQLPYDRNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST
      190     200     210     220     230     240

      250     260     270     280     290     300
m121-1.pep GRELFALNWLITYLDGGENRYDVLRTLSRFTAQTVCDAYSHAAADARQMYICGGGIRNPV
g121        GRELFALNWLITYLDGGENRYDVLRTLSRFTAQTVCDAYSHAAADARQMYICGGGIRNPV
      250     260     270     280     290     300

      310     320     330     340     350     360
m121-1.pep IMADLAECFPGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
g121        IMADLAECFPGTRVSLHSTAEINLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
      310     320     330     340     350     360

m121-1.pep XAGYYYYX
g121        GAGYYYYX

```

- 91 -

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

```
a121-1.seq
1   ATGGAACAC  AGCTTTACAT  CGGCATCATG  TCGGGAACCA  GCATGGACGG
51  GCGGGATGCC  GTACTGATAC  GGATGGACGG  CGGCAATGG  CTGGGCGCGG
101 AAGGGCAGCG  CTTTACCCCC  TACCCGCGCA  GGTTACGCCG  CAATTTGCTC
151 GATTTCAGAG  ACACAGGCGC  GGACGAACCT  CACCGCAGCA  GGATGTGTCT
201 GCAAGAACTC  AGCCGCGCTG  ACGCGCAAC  CGCCGCGGAA  CTGCTGTGCA
251 GTCAAAACCT  CGCGCGCTCG  GACATTAACG  CCCTCGGCTG  CCACGGGCAA
301 ACCGTCAGAC  ACGCGCGCGA  ACACAGTTAC  AGCGTACAGC  TTGCGGATTT
351 GCGGCTGCTG  CGGGAACGGA  CTCAGATTTT  TACCGTCGGC  GACTTCGCGA
401 GCGGCGACCT  TGCGGCGCGC  GCACAAGGCG  CGCGCTCGT  CCCGCGCTTT
451 CACGAAGCCC  TGTTCGCGCA  CGACAGGGAA  ACACGCGCGG  TACTGAACAT
501 CGCGGGGATT  GCAACATCA  GGGTACTCCC  CGCCGACGCA  CCGGCTTCG
551 GCTTCGACAC  AGGACCGGGC  AATATGCTGA  TGGACGCGTG  GATGACGGCA
601 CACTGGCAGC  TTCCTTACGA  CAAAACGGT  GCAAGGCGG  CACAAGGCAA
651 CATATTGCGG  CAATGCTCG  ACAGGCTGCT  CGCCACCGG  TATTTGCGAC
701 AACCCACGCC  TAAAGCAGC  GGGCGGAA  TGTTTGCCCT  AAATTGGCTC
751 GAAACCTACC  TTGACGCGG  CGAAACCGA  TACGAGCTAT  TGGGACGCT
801 TTCCGATTC  ACCGCGCAA  CGGTTTCGA  CGCGCTCTCA  CACGCGCGG
851 CAGATGCCG  TCAATGTGAC  ATTTGCGGCG  CGCGATCCG  CAATCTGCTT
901 TTAATGCGG  ATTTGCGAGA  ATGTTGCGG  ACACGCTGCT  CCTGCGACG
951 CACCCCGGAA  CTGAACCTCG  ATCCGCAATG  GGTAGAAGCC  GCGCGTTCT
1001 CATGGATGCG  GCGGTGTTGG  CTCACCGCA  TTCCGGTAG  TCOCACAAA
1051 GCAACCGCG  CATCCAAAC  GTGTATCTG  GCGCGGGAT  ATTATTATT
1101 A
```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

```
a121-1.pep
1   METQLYIGIM  SCTSMGDADA  VLIRMDGGKW  LGAEHAFTP  YPGLRLRKLL
51  DLQDTGADEL  HRSRMLSQEL  SRLYAQTAAE  LLCQNLAPE  DITALGCHGQ
101 TVRHAPHESY  SVQLADLPLL  AERTQITVVG  DFRSRDLAAG  GQAPLVPFA
151 HEALFRDDRE  TRAVLNIGGI  ANISVLPPDA  PAFGFDTPG  NMLMDAWMQA
201 HWQLPYDKNG  AKAAQGNILP  QLLDRLLAHP  YFAQPHKST  GRELFALNWL
251 ETYLDGGENR  YDVLRTLSRF  TAQTVFDVAV  HAAADARQWY  ICGGGIRNPV
301 LMADLAECFG  TRVSLHSTAE  INLDPQWVEA  AAFAWMAACW  VNRIPGSPHK
351 ATGASKPCIL  GAGYYY*
```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEHAFTPYPGLRLRRQLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEHAFTPYPGLRLRRQLLDLQDTGADEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSQNLAPELADLALGCHGQTVRHAPHESYQLADLPLL					
a121-1	HRSRMLSQELSRLYAQTAAELLCSQNLAPELADLALGCHGQTVRHAPHESYQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTQITVGDFFRSRDLAAGGQAPLVPFAHEALFRDRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQITVGDFFRSRDLAAGGQAPLVPFAHEALFRDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHKST					
a121-1	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHKST					
	190	200	210	220	230	240

- 92 -

```

                250      260      270      280      290      300
m121-1.pep    GRELFALNWLETYLDGGENRYDVLRLSLSRFTAQTVCDAVSHAAADARQMYICGGGIRNPV
                |||
a121-1        GRELFALNWLETYLDGGENRYDVLRLSLSRFTAQTVFDAVSHAAAADARQMYICGGGIRNPV
                |||
                250      260      270      280      290      300

                310      320      330      340      350      360
m121-1.pep    LMADLAECGFTRVSLHSTADLNLDPQWVEAAFXFAWLAACWINRIGPSPHKATGASKPCIL
                |||
a121          LMADLAECGFTRVSLHSTADLNLDPQWVEAAAFAWMAACWNRIGPSPHKATGASKPCIL
                |||
                310      320      330      340      350      360

m121-1.pep    XAGYYYY
                |||
a121          GAGYYYY

```

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

```

m128.seq      (partial)
1  ATGACTGACA ACGCACTGCT CCATTGGGG GAAGAACCCC GTTTTGATCA
51 AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATCGCGAAG
101 CGCGGAAACA AATCGCGCC ATCAAAGCCC AAACGCACAC CGGCTGGGA
151 AACACTGTCG AACCCCTGAC CGGCATCACC GAACGCGTGC GCAGGATTTTG
201 GGGCGTGTGT TCGCACTCA ACTGCGTGC CGACACGCCC GAACTGCGCG
251 CGCTCTATAA CGAACTGATG CCGGAATCA CGCTCTCTT CACGAAATC
301 GCACAAGACA TCGAGCTGTA CAACCGCTTC AAACCATCA AAAATTCCCC
351 CGAATTGCAC ACCCTCTCCC CCGCACAAA AACCAAACTC AACCAC
1  TACGCCAGCG AAAAACTCGC CGAAGCCAAA TACGCGTCA GCGAAACGGA
51 wGTCAAAAAA TayTCCCyG TCGGCAwGT ATTAAACGGA CTGTTGCCCC
101 AAmTCAAAAA ACmTACGCG ATCGGAATTA CCGAAAAAC yGTCCCCGTC
151 TGGCACAAAG ACGTGCCTA TTKTGAATTG CAACAAAACG GCGAmCCAT
201 AGCGCGGCTT TATATGGATT TGTACGCACG CAGAGGCAAA CGCGCGCGCG
251 CGTGGATGAA GCACTACAAA GGCCGCGGCC GTTTTTCAGA CGCACGCTG
301 CAAYTGCCCA CGGCCTACCT CGTCTGCAAT TTGCGCCCAC CCGTCGGGCG
351 CAGGGAAGCC CGCYTGAGCG ACGACGAAAT CCTCATCTCT TTCCAGAAA
401 CCGGACACGG GCTGCACCAC CTGCTTACCC AAGTGGACGA ACTGGGCGTA
451 TCCGCGATCA ACGCGGTAKA ATGGGAOGCG GTCCGACTCG CCAGCCAGIT
501 TATGGAAAAT TTGCTTTGGG AATACAATGT GTTGGCACAA mTGTCACGCC
551 ACGAAGAAAC CGGcgTTCCC yTGCCGAAAG AACTCTtsGA CAJAwTGCTC
601 GCCGCCAAAA ACTTCCAAsG CGGCATGTT yTsGTCGCG AAwTGGAGTT
651 CGCCCTCTTT GATATGATGA TTTACAGGA AGACGACGAA GGCCGCTCTGA
701 AAAACTGSCA ACAGGTTTTA GACACGCTG GCAAAAAAGT CGCGCTCATC
751 CAGCGCGCCG AATACACCGC CTTCGCTTG AGCTTCGCGC ACATCTTCGC
801 AGCGGCTAT TCGCGAGCTn ATTACAGCTA CGCGTGGCGG GAAGTATTGA
851 GCGCGGACGC ATACGCCGCC TTGAAGAAA GCGACGATGT CGCGCGCACA
901 GGCRAACGCT TTTGGCAGGA AATCTCGCC GTCGGGnAT CGCGCAGCGG
951 nGCAGAATCC TTCAAAGCTT TCGCGGCGCG CGAACCGAGC ATAGACGCAC
1001 TCTTGCGCCA CAGCGGTTTC GACAACGCGG TCTGA

```

This corresponds to the amino acid sequence &lt;SEQ ID 51; ORF 128&gt;:

```

m128.pep      (partial)
1  MTDNALHLHG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTWA
51 NTVEPLTGIT ERVGRIGGVV SHLNCVADTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSPAQKTKL NH

//

1  YASEKLREAK YAFSETXVKK YFFVGVXLNG LFAQXKKLYG IGFTEKTVPV

```

- 93 -

```

51  WHKDVRYXEL  QQNGEXIQGV  YMDLYAREGK  RGGAWMNDYK  GRRRFSDGTL
101  QLPTAYLVCN  FAPPVGGREA  RLSHDEILIL  FHETGHGLHH  LLTQVDELGV
151  SGINGVXWDA  VELPSQPMEN  FVWEYNVLAQ  XSAHEETGVP  LPKELKDXL
201  AAKNFQXGMF  XVRQXEFALF  IMMIYSDEDE  GRLKNWQVVL  DSVRRKKVAI
251  QPPPEYNFAL  SPGHIFAGGY  SAAXYSYAWA  EVLSADAYAA  FEESDDVAAT
301  GKRFWQEILA  VGXSRSGAES  FKAFRGREPS  IDALLRHSGF  DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

```

g128.seq
1  atgattgaca  acgCactgct  ccacttgggc  gaagaaccCC  GTTTTaatca
51  aatccaaacc  gaagACatca  AACCGGCGT  CCAAAACCGC  ATGCGCGAAG
101  CGCGCGGACA  AATCGCGGCC  GTCAAAAGCG  AAACGCACAC  CGGCTGGGCG
151  AACACCGTGG  AGGTCCTGAC  CGGCATCACC  GAACGCGTCG  GCAGGATTGG
201  GGGCGTGGTG  TCCCATCTCA  ACTCGGTCGT  CGACACGCCC  GAAGTGGGCG
251  CGCTCTATAA  GGAACCTGAT  CCGTGAATCA  CGGTCTTCTT  CACCGAAATC
301  GGACAAGACA  TCGAATCTGA  CAACCGGCTT  AAAACCATCA  AAAATTCCCC
351  CGAATTGGCA  ACGCTTTCCC  CGGCACAAAA  AACCAAGCTC  GATCACGACC
401  TGCAGCATTT  CGTATTGAGC  GGGCGGGAAC  TGCGCGCCGA  ACGGCAGGCA
451  GAACTGGCAA  AACTGCAAAC  CGAAGGCGCG  CAACTTTCOG  CCAAATCTCT
501  CCAAAACGTC  CTAGACGCGA  CGACGCGGTT  CGGCATTTAC  TTGACGATG
551  CGCACCGGCT  TGCGCGCATT  CCGAAGAGCG  CGCTGCCCAT  GTTTGCGCGC
601  GCGCGCGCAA  GCGAAGGCAA  AACAGGTTAC  AAAATCGGCT  TGCAATTTC
651  GCACCTACCTT  GCGGTTATCC  AATACGCGCG  CAACCGGAA  CTGCGGAAAC
701  AAATCTACCG  CGCTACGTT  ACCGCTGCCA  GCGAATTTTC  AAACGACGCG
751  AAATTTGACA  ACACCGCCAA  CATCGACCGC  ACGCTCGAAA  ACGCATTGAA
801  AACCGccaaa  CTGCTCGGCT  TTAATAATTA  CGCGGAATTG  TCGCTGGCAA
851  CCAAAATGGC  GGACACGCGC  GAACAGGTTC  TAAACTTTCT  GCACGACCTC
901  GCGCGCGCGC  CCAAACCTTA  GCGCGAAAAA  GACCTCGCGC  AAGTCAAAGC
951  CTTGCGCGCG  GAACACCTCG  GTCTCGCGGA  CCGCGAGCGC  TGGGACTTGA
1001  GCTACGCGCG  GGAATAAATG  CGCGAAGCCA  AATACGCTAT  CAGCGAAACC
1051  GAAGTCAAAA  AATACTTCCC  CGTGGGCAAA  GTTCTGGCAG  GCCTGTTCGC
1101  CCAATCAAAA  AACTCTACG  GCATCGGATT  CGCGAAAAAA  ACGGTTCCCG
1151  TCTGGCACAA  AGACGTGGCG  TATTITGAAT  TGCAACAAAA  CGGCAAAACC
1201  ATCGCGCGCG  TTTATATGGA  TTTGTACGCA  CGCGAAGCCA  AACGCGGGCG
1251  CGCGTGGATG  AACGACTaca  AAGGCCGCGC  CGGCTTTGCG  GACGgcacGC
1301  TGCAACTGCC  CACCGCTAC  CTGCTGTGCA  ACTTCGCCCC  GCGCGTGGCG
1351  GGCAAGAAG  CGCGTTTAAG  CCACGAAGAA  ATCTCACCCC  TCTTCCACGA
1401  AacCGGCCAC  GGAATGCAAC  ACCTGCTTAC  CCAAGTGGAC  GAAGTGGGCG
1451  TGTGCGGCAT  CAacggcgta  GAATGGGACG  CGGTGGAAGT  GCCGCGCCAG
1501  TTTATGGAAA  ACTTGGTTTG  GGAATACAAT  GTATTGGCAG  AAGTGTCCGC
1551  CACGAAGAAA  AacggCGGAG  CCGTGGCGAA  AGAAGCTTTC  GACAAATGCG
1601  TgcCGCCAA  AACTTCCAG  CGCGGTATGT  TCTCTGTCCG  GCAATGGAG
1651  TTGCGCTCT  TCGATATGAT  GATTTCAGT  GAAAGCGAGC  AATGCGCTCT
1701  GAAAACTGG  CAGCAGGTTT  TAGACAGGTT  GCGCAAGAAA  GTcGCGTCA
1751  TCCAACGCGC  CGAATACAA  CGCTTGGCAG  ACAGCTTTCG  CCacatctCT
1801  GCcggcGGCT  ATTCCGCGAG  CTAATTACAGC  TAGCATGGTG  CCGAAGTCTC
1851  cAGCACCGAT  GCCTACGCGC  CTTTGAAGA  AAGcGACgac  gtccGCGCCA
1901  CAGGCAAAAG  CTTCTGGCAA  GAAAcctctg  cgtcgggcgg  ctCCGCGACG
1951  gogCGGAAT  CTTTCAAAG  CTTTCGCGGA  CGCGAACGAC  GCATAGACGC
2001  ACTGCTGCGC  Caaaggcggt  TGACAACGCG  gGCTtga

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

```

g128.pap
1  MIDNALLHLG  EEPFRNQIQT  EDIKPAVQTA  IAEARGQIAA  VKAQHTGTWA
51  NTVRLTGIT  ERVGRIGGVV  SHLNSVVDPT  ELRAVYNELM  PEITVFPTFI
101  GQDIELYNRF  KTIKNSPEFA  TLPSPAKTKL  DHDLRDFVLS  GAELPPERQA
151  ELAKLQTEGA  QLSAKFSQNV  LDATDAFGIY  FDAAAPLAGI  PEDALAMFAA
201  AAQSEGGTGY  KIGLQIPHYL  AVIQVANGRE  LREQIYRAYV  TRASELSNDG

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- 94 -

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251 KFDNTIANIDR TLENALKTAK LLGPKNYAEL SLATKQADTP EQVLNLFHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET
351 EVKYPFPVKG VLAGLFAQIK KLYGIGFAEK TVPVVHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGAWM NDYKGRRRFA DGTQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGLNGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGEPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS ESDECLKNW QQVLSVRKE VAVIQPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSTD AYAAFESDD VAATGKRFWQ EILAVGGGSR
651 AAESEFKAFRG REPSIDALLR QSGFDNAA*

```

ORF 128 shows 91.7% identity over a 475 aa overlap with a predicted ORF (ORF 128.ng) from *N. gonorrhoeae*:

```

ml28/g128
      10      20      30      40      50      60
g128.pep MIDNALHLGEEPRFNQIQTEDIKPAVQTAIAEARGQIAAVKAQTHGTWANTVERLTGIT
      |||
ml28     MTDNALHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQTHGTWANTVEPLTGIT
      10      20      30      40      50      60

      70      80      90      100     110     120
g128.pep ERVGRINGVSHLMSVVDTPELRAVYNELMPEITVFFTEIQQDIELYNRFKTIKNSPEFA
      |||
ml28     ERVGRINGVSHLMSVVDTPELRAVYNELMPEITVFFTEIQQDIELYNRFKTIKNSPEFD
      70      80      90      100     110     120

      130     140     150     160     170     180
g128.pep TLSPAQKTKLDHDLRDFVLSGAELPPERQAEALAKIQTEGAQLSAKFSQNVLDATDAFGIY
      |||
ml28     TLSPAQKTKLNH
      130

//

      340     350     360
g128.pep YAGEKLREAKYAFSTETEVKKYFPVGVKVLG
      |||
ml28     YAGEKLREAKYAFSTETEVKKYFPVGVKVLG
      10      20      30

      370     380     390     400     410     420
g128.pep LFAQIKKLYGIGFAEKTVPVHKDVRYPFELQONGKTIGGVYMDLYAREGKRGGAWMNDYK
      |||
ml28     LFAQXKKLYGIGFTKTVPVVHKDVRYPFELQONGKXIGGVYMDLYAREGKRGGAWMNDYK
      40      50      60      70      80      90

      430     440     450     460     470     480
g128.pep GRRRFADGTQLQPTAYLVCFNFPVVGKKEARLSHDEILTLFHETGHGLHLLTQVDELGV
      |||
ml28     GRRRFSDGTQLQPTAYLVCFNFPVVGKKEARLSHDEILTLFHETGHGLHLLTQVDELGV
      100     110     120     130     140     150

      490     500     510     520     530     540
g128.pep SGINGVWDAVELPSQPMENFVWEYNVLAQMSAHEETGEPLPKELFKMLAAKNPQGMF
      |||
ml28     SGINGVWDAVELPSQPMENFVWEYNVLAQMSAHEETGVPLPKELXDKMLAAKNPQGMF
      160     170     180     190     200     210

      550     560     570     580     590     600

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- 95 -

```

g128.pep      LVRQMEFALFDMMIYSESDCRLKNWQVLDVSRKEVAVIQPPPEYNRFANSFGHIFAGGY
                |||
m128          XVRQXSEFALFDMMIYSESDDEGRLLKNWQVLDVSRKKVAVIQPPPEYNRFALSFHIFAGGY
                |||
                220      230      240      250      260      270

                610      620      630      640      650      660
g128.pep      SAGYYSYAWAEVLSTDAYAAFEESDDVAATGKRFWQIEILAVGSSRSAESFKAFRGREPS
                |||
m128          SAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQIEILAVGXSRSGAESFKAFRGREPS
                |||
                280      290      300      310      320      330

                670      679
g128.pep      IDALLRQSGFDNAAX
                |||
m128          IDALLRHSFGFDNAVX
                |||
                340

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

```

a128.seq
1      ATGACTGACA  ACGCACTGCT  CCATTGGGGC  GAAGAACCCC  GTTTTGATCA
51     AATCAAAACC  GAAGCACTCA  AACCGCCCT  GCAACCGGCT  ATTGCGGAAG
101    CGCGGACACA  AATCGCGCGC  ATCAAAAGCC  AAACGCAACG  CGGCTGGGCA
151    AACACTGTGC  AACCCCTGAC  CGGCTACACC  GAAGCGGTGC  GCAGATTFTG
201    GGGCGTGTG  TCGCACTCTA  ACTCCGTAC  CGACAGCGCC  GAAGTGGGCG
251    CGCGCTACAA  TGAATTAAATG  CCGGAATAT  CGGTCTCTTT  CACGGAATC
301    GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACCATCA  AAAACTCCCC
351    CGAGTTCGAC  ACCCTCTCCC  ACGCGCAAAA  AACCAAACTC  AACACGATC
401    TGGCGGATTT  GGTCTCTCAG  GGCGCGGAAC  TGCGGCCGGA  ACAGCAGGATC
451    GAATTGGCAA  AACTGCAAA  CGAAGCGCG  CAATCTTCGG  CCAAAATCTC
501    CCAAAACGTC  CTAGACGCGA  CCGAGCGGTT  CGGCAATTAC  TTTGACGATG
551    CGCACCGCGT  TGC CGCGATT  CCGCAAGAC  CGCTCGCCAT  GTTTGCGCGT
601    GCGCGCGAAA  GCGAAGGCAA  AACAGGCTAC  AAAATCGGTT  TGCAGATTCC
651    GCACTACCTC  GCGGCTCATC  AATACGCGGA  CAACGCGAAA  CTGCGCGAAC
701    AARTCTACCG  CGCTACGTT  ACCCGCGCCA  GCGAGCTTTC  AGACGACGGC
751    AARTTCGACA  ACACCGCCAA  CATCGACGCG  ACGCTCGAAA  ACGCCTCGCA
801    AACCGCCAAA  CTGCTCGGCT  TCAAAAAC  CGCGCAATTG  TCGCTGGCAA
851    CCAAAATGGC  GGACACCCCC  GAACAGTTT  TAAACTTCTC  GCACGACCTC
901    GCGCGCGCGC  CCAAAACCTA  CGCGAAGAAA  GACCTCGCCG  AAGTCAAAGC
951    CTTCGCGCGC  GAAAGCTFCG  GCCTCGCGGA  TTTGCAACCG  TGGGACTFTG
1001   GUTACGCGCG  GAAAAAAC  CGCGAAGCCA  AATACGATT  CAGCGAATCC
1051   GAAGTCAAAA  AATACTTCCC  CGTGCAGAAA  GATTAAAGC  GACTGTCGC
1101   CCAAAATCAA  AAACCTACG  GCATCGGATT  TACCGAAAA  ACCGTCGCCG
1151   TCTGCGCAAA  AGACGTGCGC  TATTTGAAT  TGCAACAAA  CGGCGAATCC
1201   ATAGCGCGCG  TTTATATGGA  TTTGTACGCA  CGGGAAGGCA  AACGCGCGCG
1251   CGCGTGGATG  AAGGACTACA  AAGGCGCGCG  CGGTTTTCGA  GACGCGACGC
1301   TGCAACTGCC  CACCGCTTAC  CTGCTGCGCA  ACTTCACCCC  GCGCGTGGCG
1351   GGCAAGGAAG  CCGGCTTGAG  CCATGACGAA  ATCCTCACCC  TCTTCCACGA
1401   AACCGGACAC  GGCCTGCACC  ACCTGCTTAC  CCAAGTCGAC  GAAGTGGGCG
1451   TATCGGCGAT  CAACGGCGTA  GAATGGGACG  CAGTGGAACT  GCGGCTCGAG
1501   TTTATGGAAA  ATTTCGTTTG  GGAATACAAT  GTCTTGGCGC  AAATGTCGCG
1551   CCACGAGAAA  ACGCGGTTTC  CCTCGCGGAA  AGAATCTCTC  GACAAAAATG
1601   TCGCGGCCAA  AAATCTCCAA  CGCGGAATGT  TCCTCGTCCG  CCAAAATGGAG
1651   TTGCGGCTCT  TTGATATGAT  GATTACAGC  GAGACGAGC  AAGCGGCTCT
1701   GAARAACCTG  CAACAGCTTT  TAGACAGCT  GCGCAAGAAA  GTCCGCGTGC
1751   TCGCGCGCGC  CGAATACAA  CGCTTCGCA  ACAGCTTCGG  CCACATCTTC
1801   CAGCGCGGCT  ATTCCGAGG  CTATTACAGC  TAGCGTGGG  CGGAATGATT
1851   GAGCGCGGAC  GCATACGCGC  CCTTTGAAGA  AAGCGAGAT  GTCCGCGGCA
1901   CAGCGCAACG  CTTTGGCAG  GAAATCTCTG  CGCTCGGCG  ATCGCGGACG
1951   GCGCGAGAAT  CCTTCAAGC  CTTCCGCGGA  CGGAAACGGA  GCATAGACGC
2001   ACTCTTGCGC  CACAGCGGCT  TCGACAAACG  GCCTTGA

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- 96 -

This corresponds to the amino acid sequence &lt;SEQ ID 55; ORF 128.a&gt;:

```

a128.pep
1   MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQTHTGWA
51  NTVEPLTGIT ERVGRINGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAQ LLGFKNYAEL SLATKMADTP EQVLNLFHLD
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLPQ WDLGYAGEKL REAKYAFSET
351 EVKRYFPVGG VLNGLFAQIK KLYGIGFTEK TVPVWHKDVR YFELQQNGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTQLQLPTAY LVCNFTFPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV ENDAVELSPQ
501 FMENFVWEYN VLAQMSAHEE TGVFLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFMMIYS EDEGRKKNW QVGLDSVRKE VAVVRPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ ETLAVGGSR
651 AAESEKAFKG REPSIDALLR HSGFDNAA*

```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```

m128.pep      10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAATKAQTHTGWANTIVEPLTGIT
|||||
a128           10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAATKAQTHTGWANTIVEPLTGIT
|||||

m128.pep      70      80      90      100     110     120
ERVGRINGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
|||||
a128           70      80      90      100     110     120
ERVGRINGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
|||||

m128.pep      130
TSPAQKTKLNH-----
|||||
a128           130     140     150     160     170     180
TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY
|||||

m128.pep      -----
a128           190     200     210     220     230     240
FDDAAPLAGIPEDALAMFAAAQSEGKTGYKIGIQTIPHYLAVIQYADNRKLEQIYRAYV
|||||

m128.pep      -----
a128           250     260     270     280     290     300
TRASELSDDGKFDNTANIDRTLENALQTAQKLLGFKNYAELSLATKMADTPEQVLNLFHLD
|||||

m128.pep      -----
a128           310     320     330     340     350     360
ARRAKPYAEKDLAEVKAFARESLGLADLPQWDLGYAGEKLREAKYAFSETEVKRYFPVGG
|||||

m128.pep      160     170     180     190     200     210
VLNGLFAQXKIKLYGIGFTEKTPVPVWHKDVRXYELQQNGETIGGVYMDLYAREGKRGGAWM
|||||
a128           160     170     180     190     200     210
VLNGLFAQIKKLYGIGFTEKTPVPVWHKDVRXYELQQNGETIGGVYMDLYAREGKRGGAWM
|||||

m128.pep      220     230     240     250     260     270
NDYKGRRRFS DGTQLQLPTAYLVCNFTFPVGGREARLSHDEILILFHETGHGLHLLTQVD
|||||

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- 97 -

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|||||
a128 NDYKGRRRFSDGTLQLPTAYLVCFNFTFPVGGKEARLSHDEILTLFHEHGLHLLTQVD
      430      440      450      460      470      480

280      290      300      310      320      330
m128.pep ELGVSGINGVXWDAVELFSQFMENFVWEYNVLAKXSAHEETGVPLFKELXDKXLAANKFO
|||||
a128 ELGVSGINGVWDAVELFSQFMENFVWEYNVLAKXSAHEETGVPLFKELFKMLAANKFO
      490      500      510      520      530      540

340      350      360      370      380      390
m128.pep XGMEKVRQXEFALFDMMIYSEDEGRLLKNQOVLDSVRKQVAVITQPEYNRFALSFGHIF
|||||
a128 RGMFLVRQMEFALFDMMIYSEDEGRLLKNQOVLDSVRKEVAVVRPPEYNRFANFSFGHIF
      550      560      570      580      590      600

400      410      420      430      440      450
m128.pep AGGYSAXXSYAWAEVLADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRG
|||||
a128 AGGYSAGYSSYAWAEVLADAYAAFEESDDVAATGKRFWQEILAVGGSRSAESFKAFRG
      610      620      630      640      650      660

460      470
m128.pep REPSIDALLRHSGFDNVAX
|||||
a128 REPSIDALLRHSGFDNAAX
      670

```

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 56>:

```

m128-1.seq
1 ATGACTGACA ACGCACTGCT CCATTGGGG GAAGAACCOC GTTTTGATCA
51 AATCAAAACC GAAGACATCA AACCOCGCC GCACAAACGCC ATCGCCGAAG
101 CGCGCGAACA AATCGCGGCC ATCAAAAGCC AAACGCACAC CGGCTGGGCA
151 AACACTGTGC AACCCTGAC CGGCATCACC GAACGCGTGC GCAGGATTG
201 GGGCGTGGTG TCGCACTCA ACTCGCTCG CGACACGCC GAACGCGGC
251 CGCTCTATAA GAACTGATG CCGGAAATCA CGCTCTCT CACCGAAATC
301 GGACAAGACA TCGAGCTGA CAACCGCTC AAAACATCA AAAATTCCOC
351 CGAATTGAC ACCCTCTCC CGGCACAAA AACCAACTC AACCGATC
401 TGCAGGATT GTCTCTCAG GCGCGGAAC TGCCGCCGC ACAGCAGGA
451 GAACTGGCAA AACTGCAAC CGAAGCGCG CAACCTTCCG CCAAAATCTC
501 CCAAAAGCTC CTAGACGGA CCGAAGCGT CGGCATTAC TTGACGATG
551 CGCACGCGCT TGCGCGCAT CCGAAGAGC CGCTGCGCAT GTTTGCGGCC
601 GCGCGCGAAA GCGAAGCAA AACAGGCTAC AAATCGGCT TGCAAGATTCC
651 ACACCTACCTC GCGCTCATCC AATACGCGA CAACCGCGAA CTCGCGGAAC
701 AAATCTACCG CGCTACGTT ACCCGCGCA CGGAACCTTC AGACGACGCG
751 AAATTCGACA ACACGCGCAA CATCGACCG AGCTCGCAA AGCCTCGCA
801 AACCGCAAAA CTGCTCGCT TCAAAACTA CGCGAATTG TCGCTGGCAA
851 CCAAAATGCG GACACGCGC GAACAATTT TAACTTCTT GCACGACTC
901 GCGCGCGCG CCAACCCCTA CGCGAAAAA GACTCGCGC AAGTCAAAAC
951 CTTCGCGCGC GAAAGCGTA ACCTCGCGA TTTGCAACCG TGGGACTTGG
1001 GCTACGCGAG CGAAAACTG CGCGAAGCA AATACGCTT CACGAAAC
1051 GAAGTCAAAA AATACTTCC CGTCGCGAAA GTATTAAACG GACTGTTCCG
1101 CCAAAATCAA AACTCTACG GATCGGATT TACGAAAAA ACCGTCGCC
1151 TCTGGCACA AGACGTGCG TATTTTGAA TGCAACAAA CGCGAAGC
1201 ATAGCGCGG TTTATATGA TTGTACGCA CGCGAAGCA AACGCGCGG
1251 CGCGTGGATG AACACTACA AAGCGCGCG CCGTTTTTCA GACGCGACG
1301 TGCAACTGCC CACCGCTAC CTGCTGCA ACTTCGCCC ACCGCTCGG
1351 GGCAGGGAAG CCGCGCTGAG CCACGAGAA ATCCTATCC TCTTCCAGA
1401 AACCGGACAC GGGCTGCACC ACCTGCTTAC CCAAGTGAC GAACGGGCG

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- 98 -

```

1451 TATCCGCGCAT CAACGGCGTA GAATGGGACG CGGTGGAAGT GCCAGCCAG
1501 TTTATGGAAA ATTTGGTTTG GGAATACAAT GTCTTGGCAC AAATGTCAGC
1551 CCACGAAGAA ACCGGCGTTC CCTCGCGGAA AGAAGCTCTC GACAAAATGC
1601 TCGCGCGCAA AAAGCTTCAA CGGGCGATGT TCCTCGTCCG GCAAATGGAG
1651 TCGCGCTCTT TTGATATGAT GATTTCACAG GAAGCAGCAG AAGGCGGTCT
1701 GAAAAAGCTGG CAACAGGTTT TAGACAGCGT GCGCAAAAAA GTGCGCGTCA
1751 TCCAGCGCGC GGAATACAGG CGCTTCGCGT TGAGCTTCGG CCACATCTTC
1801 GAGGCGCGCT ATTCGCGAGG CTATTCACAG AAGCGTGGG CGGAATATT
1851 GCGCGCGGAC GCATACGCGG CCTTGAAGA AGCGACGAT GTGCGCGCA
1901 CAGGCAAGCG CTTTTGGCAG GAAATCTCGC CGCTCGCGG ATCGCGCAGC
1951 GCGGCAAGAT CTTTCAAGC CTTTCGCGCG CGCGACGCA GCATAGACGC
2001 ACTCTTGGCG CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1. pep.

```

1  MTDNALLHLG EEPFDFQIKT EDIKPALQTA IAEAREQIAA IKAQTHGWA
51  NTVEPLTGIT ERVGRWGVV SHINSVADTP ELRAVYNELM FEITVFEFEI
101 GQDIELYNRF KTAKNSPEFD TLPSPQKTKL NHDLRDPVLV GAELPFEQQA
151 ELAKLQTEGA QLSAKPSQNV LDATDARGIY FDDAAPLAGI PEDALAMFAA
201 AAQSESKTGY KIGLIQPHYL AVIQYADNRE LREQIYRAYV TRASELSDG
251 KFDNTANIDR TLANALQAK LLGFKNYAEL SLATKMAOTP EQVLFNLHDL
301 ARRAKPYAEK DLAEVKAFAR ESNLADLOLP WDLGYASEKL REAKYAFSET
351 EVKRYFPVGK VINGLFQAQK KLYGIGPTEK TVPVNKHQDV YFELQNGET
401 IGGVYMDLYA REGKRGGAWM NDYKRRRRFS DGTQLQPTAY LVNCFAPVPG
451 GREARLSHDE LILFHEFHG GLHLLTQVD ELGVSGINGV ENDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPILKPEL DKMLAAKNFP RGMFLVRQME
551 FALFDMMIYS EDDEGRLLKNW QVLDLSVRKK VAVIQPEEYN RFALSTGHIF
601 AGGYSAGYYS YAWAEVLSDA AYAAFEESDD VAATGKRFEW EILAVGGERS
651 AAESFKAFRG REPSIDALLR HSGFNNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1  ATGATTGACA ACGCACTGCT CCACCTGGCG GAAGAACCCC GTTTTAATCA
51  AATCAAAGCC GAAGACATCA AACCOCGCGT CCAAACCCGC ATCGCGGAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGCG
151 AACACCGTGC AGCGCTCTGAC CGGCATCACC GAACGCGTGC GCAGGATTTC
201 GGGCGTCTGT TCCCATCTCA ACTCGCTCGT CGACACGCCC GAAGTGGCG
251 CGCTCTATAA CGAACTGATG CCGTGAATCA CCGTCTCTTT CACGGAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGACA ACGCTTTCCC CGGCACAAAA AACCAAGCTC GATCAGGACC
401 TGGCGGATTT CGTATTGAGC GGGCGGGAAC TGCGGCCGGA ACGGCAGGCA
451 GAAGTGGCAA AACTGCAAA CCGAAGCGCG CAAGCTTCCG CCAAAATCTC
501 CCAAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTGACGATG
551 CCGCACCGCT TCGCGGCAAT CCGCAAGCAG CGCTCGCCAT GTTTCGCGCC
601 GCGCGGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCATACCTTT GCGCTTATCC AATACGCGCG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCTACGTT ACCCGTGCCA CGCAACTTTC AAACGACGG
751 AAATTCGACA ACACCGGCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGCAAAA CTGCTCGGCT TTAATAATTA CGCGCAATTG TCGCTGGCAA
851 CCAAAATGGC GGACAGCGCC GAACAGGTTT TAAACTTCCT GCACAGCTC
901 GCGCGCGCGC CCAAAACCTA CGCCGAAAAA GACCTCGCGC AAGTCAAAGC
951 CTTGCGCGCG GAACAACCTG GTCTCGCGCA CCGCGACGCG TGGGACTTGA
1001 GCTACGCGCG GAAAAAAGTC CGCGAAGCCA AATACGCAAT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTGGCGAAA GTTCTGGCAG GCCTGTTCCG
1101 CCAAAATCAAA AAATCTCAAC GCATCGGATT CCAGCAAAAA ACCGTTCCCG
1151 TCTGGCACAA AGACGTGGCG TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGGCGGCG TTTATATGGA TTTGTAACGA CGCGAAGGCA AACGCGGCG
1251 CGCTGGGATG AACGACTACA AAGCGCGCGC CGCTTTGGCC GACGCGCAGC
1301 TCGAATGCGC CACGCTCTAC CTGCGTGTGA ACTTCGCGAG CCCTCGCGCG
1351 GCGAAACGAG CGCGTTTAA CCGACGAGAA ATCTCAACCC TCTTCCAGCA
1401 AACCGGCGAC GCACTGCAAC ACTGCTTTAC CCAAGTGGAC GAAGTGGCG
1451 TGTCGGCGAT CAACGGCGTA AAA

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- 99 -

This corresponds to the amino acid sequence &lt;SEQ ID 59; ORF 128-1.ng&gt;:

g128-1.pep (partial)  
 1 MIDNALLHLG EEPFRNQIKT EDIKFAVQTA IAEARGQIAA VKAQHTHTGWA  
 51 NTVERLTGIT ERVGRWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI  
 101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA  
 151 ELAKLOTTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA  
 201 AAQSEGKTCY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG  
 251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMDPT EQVLNFIHLDL  
 301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET  
 351 EVKKYFPVGG VLAGLFAQIK KLYGIGFAEK TVPVNKHVDV YFELQNGKGT  
 401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQPTAY LVCNFAPFVG  
 451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

**m128-1/g128-1** ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa overlap

	10	20	30	40	50	60
g128-1.pep	MIDNALLHLG	EEPRFNQIKT	EDIKFAVQTA	IAEARGQIAA	VKAQHTHTGWA	NTVERLTGIT
m128-1	MTDNALLHLG	EEPRFDQIKT	EDIKPAQTA	IAEAREQIAA	VKAQHTGWT	VEPLTGIT
	10	20	30	40	50	60
	70	80	90	100	110	120
g128-1.pep	ERVGRWGVV	SHLNSVVDTP	PELRAVYNELM	PEITVFFTEI	IGQDIELYNRF	KTIKNSPEFA
m128-1	ERVGRWGVV	SHLNSVADT	PELRAVYNELM	PEITVFFTEI	IGQDIELYNRF	KTIKNSPEFD
	70	80	90	100	110	120
	130	140	150	160	170	180
g128-1.pep	TLSPAQKTKL	DHDLRDFVLS	GAELPPERQA	ELAKLOTTEGA	QLSAKFSQNV	LDATDAFGIY
m128-1	TLSPAQKTKL	NHDLRDFVLS	GAELPPERQA	ELAKLOTTEGA	QLSAKFSQNV	LDATDAFGIY
	130	140	150	160	170	180
	190	200	210	220	230	240
g128-1.pep	FDDAAPLAGI	PEDALAMFAA	AAQSEGKTCY	KIGLQIPHYL	AVIQYAGNRE	LREQIYRAYV
m128-1	FDDAAPLAGI	PEDALAMFAA	AAQSEKTCY	KIGLQIPHYL	AVIQYAGNRE	LREQIYRAYV
	190	200	210	220	230	240
	250	260	270	280	290	300
g128-1.pep	TRASELSNDG	KFDNTANIDR	TLENALKTAK	LLGFKNYAEL	SLATKMDPT	EQVLNFIHLDL
m128-1	TRASELSDDG	KFDNTANIDR	TANALQTA	LLGFKNYAEL	SLATKMDPT	EQVLNFIHLDL
	250	260	270	280	290	300
	310	320	330	340	350	360
g128-1.pep	ARRAKPYAEK	DLAEVKAFAR	EHLGLADPQP	WDLSYAGEKL	REAKYAFSET	EVKKYFPVGG
m128-1	ARRAKPYAEK	DLAEVKAFAR	ELNLDLQ	WDLGYASEKL	REAKYAFSET	EVKKYFPVGG
	310	320	330	340	350	360
	370	380	390	400	410	420
g128-1.pep	VLAGLFAQIK	KLYGIGFAEK	TPVPVNHKDV	YFELQNGKGT	IGGVYMDLYA	REGKRGGAWM
m128-1	VNLGLFAQIK	KLYGIGFAEK	TPVPVNHKDV	YFELQNGKGT	IGGVYMDLYA	REGKRGGAWM
	370	380	390	400	410	420
	430	440	450	460	470	480
g128-1.pep	NDYKGRRRF	ADGTQLQPTAY	LVCNFAPFVG	GKEARLSHDE	ILTLFHETGH	GLHLLTQVD

m128-1 NDYKRRRFS DGTQLP LTA YLV C NFA P V G G R E A R L S H D E I L I L F H E T G H G L H H L L T Q V D  
 430 440 450 460 470 480  
 g128-1.pep ELGVSGINGVK  
 490  
 m128-1 ELGVSGINGV E N D A V E L S Q F M E N F V W E Y N V L A Q M S A H E E T G V P L P E L F D K M L A A K N F  
 490 500 510 520 530 540

128-1.seq

1	ATGACTGACA	ACGCATGCT	CGATT7GGGC	GAGAACACCC	GT7TTGATCA
5	AATCAAACAC	GAAAGCATCA	AACCCGCCCT	CGAAACCGCC	AT7TCCGGAAG
101	CGCGCGAACAA	ATGCTCGGCC	ATFCAAGGCTT	AACACGACAT	CGGTGGGCAAC
151	AACACTGTGCG	AACCTCTGAC	CGGCACTCAC	GACACGGCTG	CGAGGAT7GCG
201	GGGGCTGGTG	TGCGACTCTA	ACTCTCGTCA	CGACACGGCC	GAACTGTCCGG
251	CGCGCTACAA	TGAAT7AATG	CGCGAAATTA	CGGTCT7CTT	CGCGGAATCT
301	GGACAACTGCA	TGCGAGTCTT	CAACTCGCTT	AAACCATCTA	AAACACTCCCC
351	CGAGTTCGAC	ACCTCTTCCC	CGCGCAAAA	AAACCAACTC	AACCACTGATC
401	TGCGCTGAT7	CGTCTTCAGC	GGCGCGGCGA	TGCGCCCGCA	ACAGCAGGCA
451	GAA7TGGCAA	ATAG7GCAAC	AGAGGGGGCT	CAACTTTCGC	CCAAAT7TCT
501	CCAAAACCTG	CTGACGCGGA	CGACGGGCTT	CGGATCTTCG	TTTGACGATG
551	CGCGACCGCT	TGCGCGGATT	CGCGAAGACG	CGCTCGGCAT	GT7TGCGCTG
601	CGCGCGGACCA	CGGAGAGGAT	ACACGCGTAC	AAAT7GGCTT	GTTGAGAT7CC
651	CGACTATGCG	CGCGCTATCC	CGCGGCGGCA	CGGCGGCTG	CGGAGGAGTCC
701	AAATCTACCG	CGCTACTGTT	ACTCGCGCCA	CGAGACTCTG	AGACGACGCA
751	AAATCTGACA	ACACGCGCAA	CGACCGCGC	ACGCTCGAAA	AGCGCT7GCA
801	AAACCGCCAA	CTGCTCGGCT	TCMAAACTA	CGCGGAAT7T	TGCG7TGCAA
851	CCAAAATGCG	GGACACACCC	CGGCACTT7T	TAAATCTTCT	GCAGCACTCT
901	CGCGCGCGCG	CCAAACCTTA	CGCGGAAAA	GACCTCGCGC	AGCTCAAAGC
951	CT7TGCGCCG	GAAAGCGCTG	CGCT7GCGCA	TTTGACGCG	TGGGACT7TG
1001	GCTACGCGCG	CGAAAATCTG	CGCGAGCCA	ATATGACTCT	CAGCGAACTG
1051	GAA7TCAAAA	AATACT7CCC	CGGCAACCA	GTATTAAAGC	GACTCT7CCG
1101	CCAAATCAAA	AARCTCTGAC	CGATTGCGCT	TACCGAAAAA	ACCGTCCCGC
1151	T7TGGCAGCA	AGAGCTTGCG	TATT7TGAAT	TGCAACAAAA	CGCGGACAAA
1201	ATAGGCGCGC	TTTATATGGA	TT7TACGCA	CGCGGATGCA	AACCGCGCGG
1251	CGCTGGTATG	ACAGCTACTA	AAGGCGCGCG	CGGT7TTTCA	GACGCGAGTC
1301	TCGCACTCTG	CGCGCTCTAC	CTCGTCTGCA	ACTTCAACCC	CGCGCTCGCG
1351	GGCAAAAGAG	CCCGCTTGAG	CGCTACGAA	ATCTTCCACT	TCTT7CACGA
1401	AACGGGACAC	GGCTCGCACC	ACCTCTGTAC	CCAAGTGAC	GAAGCTGGCG
1451	TATTCGCAAT	CRAAGCGGCTA	GAA7TGGGAT	CAGTGGCACT	CGCCAGT7GC
1501	TT7ATGGAAGA	ATT7CT7TGT	GGATACCAAT	CT7TGGCGCG	AAATGTCCGC
1551	CCAGCAAGAA	ACCGCGG7TC	CGCTCGGCA	AGAACTCTC	GACAAAAT7G
1601	TGCGCGCCAA	AAACT7PCAA	CGCGAAAT7T	TCTCTGCTCG	CCAAAT7GAT
1651	TTGCGCTGCT	T7TATATGAT	GAT7TAAGCT	GAGACGACG	AGAGCGGCTCT
1701	GAAAT7TCTG	CGGCTGAGCA	CGCGAGAGAA	CGCGAGAGAA	CGGCTGAT7G
1751	CCGACGACGCA	CGAT7TCAAC	CGCT7CGGCA	CGGCTCTCTT	CGGCTCTCTT
1801	GAGCGGGCTC	ATT7CCGACG	CT7ATACAG	TACGCT7GTC	CGGAG7ATTG
1851	GAGGCGGACG	GCATAG7CCG	CG7T7GAGA	AGAGCAACTC	CTCGCGGCCA
1901	CAGGCAACGA	CT7TTTTCGAC	GAAATCTGAC	CG7TGGCGG	ATCGCGCACT
1951	GCGGACAGAT	CTCTCAAGCG	CGGCAACCG	CGGCAACCG	GCATAGACGG
2001	ACTCT7TGGC	CBAGCGGCTC	TGCGAC7GCG	GGCT7TGA	

a128-1.pep

1	NTDNALHLHG	EEPRFDQIKT	EDIKPAQLQT	IAEAREQIAA	IKAQHTGWA
51	NTVEPLTGIT	ERVGRIGWVV	SHLNSVDTDP	ELRAAYNELM	PEITVFFTEI
101	GDQIELYNEI	TKIKNSPEDF	TLISHAQKGL	HLNDRLDFVS	GAELPPEQQA
151	ELAKIQTEGA	QLSKASQNV	LDATATFGL	FDDAPLPLGI	PEDALAMFAA
201	AAQSSEKGTQ	KGTGLPIPHY	AVIQYADNRK	LRQIYRAYTV	TRAEVSLDDG
251	KFDNTAMNDR	TLENALQATL	LGFGKFAKIL	SLATMKMDE	QVNLNFDHGL

- 101 -

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301  ARRAKPYAEK  DLAEVKAFAR  ESLGLADLQF  WDLGYAGEKL  REAKYAFSET
351  EVKKYFFVGK  VLNLGFAQIK  KLYGIGFTEK  TVPVWHKDV  YFELQQNGET
401  IGGVYMDLYA  REGKRGGAWM  NDYKGRRRFS  DGTLLQPTAY  LVCNFTFPVG
451  GKEARLSHDE  ILTLFHETGH  GLHLLTQVD  ELGVSGINGV  EWDVAVLPSQ
501  FMENFVWEYN  VLAQMSAHEE  TGVPPLPKLF  DKMLAARNFQ  RGMFLVRQME
551  FALFDMMIYS  EDDEGLLQW  QQVLDSVRKE  VAVVRPPEYN  RFANSFGHIF
601  AGGTSAGYYS  YAAAEVLSD  AYAFAEESDD  VAATGKRFQW  EILAVGGSR
651  AAESFKAFRG  REPSIDALLR  HSCFDNA*

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**m128-1/a128-1** ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

	10	20	30	40	50	60
a128-1.pep	MTDNALLHLGEEPRFDQ	KTEDIKPALQTAIAEA	REQIAAIAKAQTH	TGWANTVEPL	TGTT	
m128-1	MTDNALLHLGEEPRFDQ	KTEDIKPALQTAIAEA	REQIAAIAKAQTH	TGWANTVEPL	TGTT	
	10	20	30	40	50	60
a128-1.pep	ERVGRINGVSHMSV	TDTPELRAAYNELM	PEITVFFTEIGQD	IELYNRFKTI	KNSPEFD	
m128-1	ERVGRINGVSHMSV	TDTPELRAAYNELM	PEITVFFTEIGQD	IELYNRFKTI	KNSPEFD	
	70	80	90	100	110	120
a128-1.pep	ERVGRINGVSHMSV	TDTPELRAAYNELM	PEITVFFTEIGQD	IELYNRFKTI	KNSPEFD	
m128-1	ERVGRINGVSHMSV	TDTPELRAAYNELM	PEITVFFTEIGQD	IELYNRFKTI	KNSPEFD	
	70	80	90	100	110	120
a128-1.pep	TLSHAQKTKLNHDL	RDFVLSGAELPPEQ	QAEAKLQTEGAQL	SAKFSQNVLDAT	DAFGYI	
m128-1	TLSPAQKTKLNHDL	RDFVLSGAELPPEQ	QAEAKLQTEGAQL	SAKFSQNVLDAT	DAFGYI	
	130	140	150	160	170	180
a128-1.pep	TLSHAQKTKLNHDL	RDFVLSGAELPPEQ	QAEAKLQTEGAQL	SAKFSQNVLDAT	DAFGYI	
m128-1	TLSPAQKTKLNHDL	RDFVLSGAELPPEQ	QAEAKLQTEGAQL	SAKFSQNVLDAT	DAFGYI	
	130	140	150	160	170	180
a128-1.pep	FDDAAPLAGIPEDAL	AMFAAAQSEKGTG	YKIGLQIPHILAVI	QYADNRKLR	EQIYRAYV	
m128-1	FDDAAPLAGIPEDAL	AMFAAAQSEKGTG	YKIGLQIPHILAVI	QYADNRKLR	EQIYRAYV	
	190	200	210	220	230	240
a128-1.pep	FDDAAPLAGIPEDAL	AMFAAAQSEKGTG	YKIGLQIPHILAVI	QYADNRKLR	EQIYRAYV	
m128-1	FDDAAPLAGIPEDAL	AMFAAAQSEKGTG	YKIGLQIPHILAVI	QYADNRKLR	EQIYRAYV	
	190	200	210	220	230	240
a128-1.pep	TRASELSDDGKF	DNTNANIDRTLAN	ALQTAKLGLGFKNY	AELS	LATKMDTPEQV	LNFLHDL
m128-1	TRASELSDDGKF	DNTNANIDRTLAN	ALQTAKLGLGFKNY	AELS	LATKMDTPEQV	LNFLHDL
	250	260	270	280	290	300
a128-1.pep	TRASELSDDGKF	DNTNANIDRTLAN	ALQTAKLGLGFKNY	AELS	LATKMDTPEQV	LNFLHDL
m128-1	TRASELSDDGKF	DNTNANIDRTLAN	ALQTAKLGLGFKNY	AELS	LATKMDTPEQV	LNFLHDL
	250	260	270	280	290	300
a128-1.pep	ARRAKPYAEKDLAE	VKFAFRESLGLADL	QFWDLG	YAGEKLREAKYAF	SETEVKKYFFVGK	
m128-1	ARRAKPYAEKDLAE	VKFAFRESLGLADL	QFWDLG	YAGEKLREAKYAF	SETEVKKYFFVGK	
	310	320	330	340	350	360
a128-1.pep	ARRAKPYAEKDLAE	VKFAFRESLGLADL	QFWDLG	YAGEKLREAKYAF	SETEVKKYFFVGK	
m128-1	ARRAKPYAEKDLAE	VKFAFRESLGLADL	QFWDLG	YAGEKLREAKYAF	SETEVKKYFFVGK	
	310	320	330	340	350	360
a128-1.pep	VLNLGFAQIKKLYG	IGFTEKTVPVWHKDV	RYFELQQNGET	IGGVYMDLYAREG	KRGGAWM	
m128-1	VLNLGFAQIKKLYG	IGFTEKTVPVWHKDV	RYFELQQNGET	IGGVYMDLYAREG	KRGGAWM	
	370	380	390	400	410	420
a128-1.pep	VLNLGFAQIKKLYG	IGFTEKTVPVWHKDV	RYFELQQNGET	IGGVYMDLYAREG	KRGGAWM	
m128-1	VLNLGFAQIKKLYG	IGFTEKTVPVWHKDV	RYFELQQNGET	IGGVYMDLYAREG	KRGGAWM	
	370	380	390	400	410	420
a128-1.pep	NDYKGRRRFS	DGTLLQPTAYLVCN	FTFPVVGKEARLSH	DEILTLFHE	TGHGLHLLTQVD	
m128-1	NDYKGRRRFS	DGTLLQPTAYLVCN	FTFPVVGKEARLSH	DEILTLFHE	TGHGLHLLTQVD	
	430	440	450	460	470	480
a128-1.pep	NDYKGRRRFS	DGTLLQPTAYLVCN	FTFPVVGKEARLSH	DEILTLFHE	TGHGLHLLTQVD	
m128-1	NDYKGRRRFS	DGTLLQPTAYLVCN	FTFPVVGKEARLSH	DEILTLFHE	TGHGLHLLTQVD	
	430	440	450	460	470	480
a128-1.pep	ELGVSGINGV	EWDVAVLPSQFMEN	FVWEYNVLAQMSA	HEETGVPLPKEL	FDKMLAARNFQ	
m128-1	ELGVSGINGV	EWDVAVLPSQFMEN	FVWEYNVLAQMSA	HEETGVPLPKEL	FDKMLAARNFQ	
	490	500	510	520	530	540
a128-1.pep	ELGVSGINGV	EWDVAVLPSQFMEN	FVWEYNVLAQMSA	HEETGVPLPKEL	FDKMLAARNFQ	
m128-1	ELGVSGINGV	EWDVAVLPSQFMEN	FVWEYNVLAQMSA	HEETGVPLPKEL	FDKMLAARNFQ	
	490	500	510	520	530	540







- 104 -

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              70      80      90      100      110      120
m206.pep      LGLIGTPYKMGSSSTATGDCSGMIQFVYKNALNVKLPRTARDMAAASRKIPDSRXKAGD
a206           LGLIGTPYKMGSSSTATGDCSGMIQFVYKNALNVKLPRTARDMAAASRKIPDSRLKAGD
              70      80      90      100      110      120

              130      140      150      160      170
m206.pep      LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNVLGAHTFFTEX
a206           LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNVLGAHTFFTEX
              130      140      150      160      170

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287

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 68>:

```

m287.seq
1      ATGTTTAAAC  GCAGCGTAAT  CGCAATGGCT  TGTATTTTGT  CCCTTTCAGC
51     CTGCGGGGCG  GCGCGTGGCG  GATCGCCCGA  TGTCAAGTCG  GCGGACACGC
101    TGTCAAAACC  TGCCGCCCTT  GTTCTTTCTG  AAAAAGAGAC  AGAGGCCAAG
151    GAAGATGGCG  CACAGGCCAG  TTCTCAAGGA  CAGGGCGCGC  CATCGGCACA
201    AGGCAGTCAA  GATATGGCGG  CGGTTTGGGA  AGAAAATACA  GGCAATGGCG
251    GTGCGGTAA  AGCGGATTAAT  CCCCAAAATG  AAGACGAGGT  GGCACAAAAA
301    GATATGCCGC  AAAATGCCGC  CGGTACAGAT  AGTTCGACAC  CGAATCACAC
351    CCGGGATCCG  AATATGCTTG  CCGGAAATAT  GGAAAAATCA  GCACCGGATG
401    CCGGGGAATC  GTCTCAGCGG  GCAAAACCA  CGGATATGGC  AAATGCGGCG
451    GACGGAAATG  AGGGGACGGA  TCGCTCGGCA  GCGGGGCAAA  ATGCGGCGAA
501    TACGGCTGCC  CAAGGTCGAA  ATCAAGCCGG  AAACAATCAA  GCGGCGCGTT
551    CTTGAGATCC  CATCCCGCGG  TCAAAACCTG  CACCTGCGAA  TGGCGGTAGC
601    AATTTTGGAA  GGGTGGATT  GCGTAATGGC  GTTTTGATTG  ACGGGCGCTC
651    GCAAAATATA  ACGTTCACCG  ACTCTAAGG  GATTTCTGT  AGTGGCAATA
701    ATTTCTTGG  TGAAGAAGTA  CAGCTAAAT  CAGATATGGA  AARATTAAGT
751    GATGCAGACA  AATAAGTAA  TTACAAGAAA  GATGGGAGGA  ATGATAAATT
801    TGTGCGTTTG  GTTGCGGATA  GTCTGCAAT  GAAGGGAATC  AATCAATATA
851    TTATCTTTTA  TAAACCTAAA  CCGACTTCAT  TTGCGCGATT  TAGGCGTTCT
901    GCACGGTCGA  GCGCGTGGCT  TCGCGCGGAG  ATGCGCGTGA  TTCCGCTCAA
951    TCAGGCGGAT  ACGTCTATTG  TCGATGGGGA  AGCGGTCAGC  CTGACGGGCG
1001   ATTCGCGCAA  TATCTTCCG  CCGCAAGGGA  ATTACCGGTA  TCTGACTTAC
1051   GGGGCGGAAA  AATTGCGCG  CGGATCGTAT  GCCTTCGTG  TTCAAGGGGA
1101   ACCGGCAAAA  GCGGAAATGC  TTGCGGGCG  GGCGGTGATC  AACGGCGAAG
1151   TACTGCATTT  CCATACGGAA  AAGCGCGCTC  CGTACCGGAC  CAGGGCGCAG
1201   TTTGCCGCAA  AAGTCGATT  CGGCAGCAAA  TCTGTGGAAG  GCATTATCGA
1251   CAGCGCGGAT  GATTTCGATA  TGGGTAAGCA  AAAATTCAA  GCGCGCATCG
1301   ATGGAAACGG  CTTTAAAGGG  ACTTGACGAG  AAAATGGCAG  CCGGGATGTT
1351   TCGGAAAGT  TTATCGGCC  GGCGCGCGAG  GAAGTGGCG  GAAATACAG
1401   ATATCGCCCG  ACAGATGCG  AAAAGGCGCG  ATTCGCGGTG  TTTGCGCGCA
1451   AAAAAGAGCA  GGAATTGA

```

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

```

m287.pep
1      MKRSVIAMA  CIFALSACGG  GGGSPDVKS  ADTLSKPAAP  VVSEKETEAK
51     EDAPQAGSQG  QGAPSAQSG  DMAAVSEENT  GNCGAVTADN  PKNEDEVAQN
101    DMPQNAAGTD  SSTFNHTPDP  NMLAGNMENO  ATDAGESSQP  ANQPDMAAQA
151    DGMQGGDDPSA  GQGQAGNTAA  QGANQAGNNQ  AAGSSDPIPA  SNFAPANGSS
201    NFGKVDLANG  VLIDGFSQNI  TLTHCKGDSC  SGNNFLDEEV  QLKSEFEKLS
251    DADKISNYKK  DGKNDKFVGL  VADSVQMKGI  NQYIIFYKPK  PTFSEFRFRS
301    ARSRRLPAE  MPLIPVQAD  TLIVDGEAVS  LTGHSGNIFA  PEGNYRYLTY

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- 105 -

351 GAELPGGSY ALRVQGEPAK GEMLAGAAYV NGEVLHFHTE NGRFPYPTGR  
 401 FPAKVDPGSK SVDGIIDSGD DLHMGTKFKF AAIDNGFPKG TWTENGSGDV  
 451 SGKIFYGPAGE EVAGKYSYRP TDAERGGFGV FAGKKEQD\*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 70>:

g287.seq  
 1 atgtttaaac gcagtgatg tgcattggct tgcatttttc ccccttcagc  
 51 ctgtgggggc ggcggtggcg gatcgccgga tgcatttcgc gcggacacgc  
 101 cgtcaaaacc ggcgcgcccc gttgttgctg aaaatgccgc ggaagggggt  
 151 ctgcggaaag aaaagaaga tgaggaggca gcggcggtg gcgcccaagc  
 201 cgatagccag gacgcaaccg ccggagaagg cagccaagat atggcgccag  
 251 tttcggcaga aaatacaggc aatggcggtg cgccaacac gcacaacccc  
 301 aaaaatgaag acgcgggggc gcaaaatgat atcgccgcaa atcgccgcca  
 351 atccgcaaat caaacaggga acaaccaacc cgccggttct tcagattccg  
 401 cccccgctc aaacccctgc cctgcgaatg gcggtagcga ttttggaagg  
 451 acgaacgtgg gcaattctgt tgcatttcgc ggaccgtcgc aaaaataaac  
 501 gttgaccacac tgtaaaaggc attcttgtaa tggtagataa ttattggatg  
 551 aagaagcacc gtcaaaatca gaatttgaaa aattaagtag tgaagaaaaa  
 601 attaaagcga ataaaaaaga cgagcaacgc gagaattttg tcggtttggt  
 651 tctgcacagg gtaaaaaagg atggaactaa caaataatc attctctata  
 701 cggaacaaac acctactcgt tctgcacagg cgaggaggtc gcttcgcgcc  
 751 gagattccgc tgattccgct caatcaggcc gatcgcctga ttgtggatgg  
 801 ggaagcggtc agcttcaggc ggcattccgc caatctcttc gcgcccgaa  
 851 ggaattacgc gtatctgact tacggggcgc aaaaattgc cgccggtatc  
 901 tatgcctctc gtgtgcaagg cgaaacggca aaaggcgaaa tgcgttggtg  
 951 cacggcgctg tacaaaggcg aagtgctgca ttcccatatg gaaaacggcc  
 1001 gtccgtaccg gtccggaggc aggtttgcgc caaaagtcca ttccggcagc  
 1051 aaatctgttg acggatttat cgacagcgcc gatgatttgc atatggtac  
 1101 gcaaaaattc aaagcgcgca tcgatggaaa cggtttaa gggacttga  
 1151 cggaataatg cggcggggat gtttccggaa ggttttacgc cccggccggc  
 1201 gaggaagtgg cgggaaaata cagctatcgc ccgacagatg ctgaaaaggg  
 1251 cggattccgc gtgtttgcgc gcaaaaaaga tcgggattga

This corresponds to the amino acid sequence <SEQ ID 71; ORF 287.ng>:

g287.pep  
 1 MFKRSVIAMA CIFELSCAGG GGGGSPDVKS ADTPSKPAAP VVAENAGEGV  
 51 LPKEKKDEEA AGGAPQALTQ DATAGEGSD MAAVSAENTG NGGAATTNDP  
 101 KNEDAGAQND MPQNAESAN CTGNQAPGAS SDSAPASNEA PANGGSDPGR  
 151 TNVNGSVVID GPSQNTILTH CKGDSNCGDN LDOEAPSKS EPEKLSDEEK  
 201 IKRYKKDEQR ENFVGLVADR VKKDGINKYI IFYDTPPPTR SARSRSLPA  
 251 EIPILFVNQA DTLIVDGEAV SLTGHSGNIF APEGNYRLVT YGAELKPGGS  
 301 YALRVQGEPA KGEMLVGTAV YNGEVLHFHM ENGRFPYPSG RFAAKVDFGS  
 351 KSVDDGIIDSG DDLHMGTKFK KAATDNGFKF GTWTENGSGD VSGRFYGPAG  
 401 EVAGKYSYRP PTDAREGGFG VFAGKKDRD\*

m287/g287 ORFs 287 and 287.ng showed a 70.1% identity in 499 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGSPDVKSADTLSKPAAPVVSE-----KTEA				
g287	MFKRSVIAMACIFPLSACGGGGGSPDVKSADTPSKPAAPVVAENAGEGVLPKEKKDEEA				
	10	20	30	40	50
	50	60	70	80	90
m287.pep	KEDAPAGSGGQGPSAPSAQSGDMAVSEENTGNGGAVTADNPKNEDEVAQNIMFQNAAGT				
g287	AGGAPQADTQD--ATAGEGSDMAVSAENTGNGGAATTDNPKNEDEVAQNIMFQNAAGT				
	70	80	90	100	110

- 106 -

```

110      120      130      140      150      160      169
m287.pep DSSTPNHPTDPNMLAGNMENQATDAGESSQFANQPDMAAADGMQGGDPSAGGGNAGNTA
g287      -----

170      180      190      200      210      220      229
m287.pep ACGANQAGNNQAAGSSDPIFASNPAFANGGNSFGRVDLANGVLIIDGFSQNTILTHCKGDS
g287      -ESANQTGNQPGAGSSDSAPASNPAFANGGSDFGRTNVGNSVVDIGFSQNTILTHCKGDS
120      130      140      150      160      170

230      240      250      260      270      280      289
m287.pep CSGNNFLDEEVQKSEFEKLSDAKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKP
g287      CNGDNLLEEAPKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKGDKGNTKIIFYTD
180      190      200      210      220      230

290      300      310      320      330      340      349
m287.pep KPTSFARFRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSNIFAPEGNYRYLT
g287      KPPT-----RSARSRRSLPAEIPLIIPVNQADTLIVDGEAVSLTGHSNIFAPEGNYRYLT
240      250      260      270      280      290

350      360      370      380      390      400      409
m287.pep YGAERKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFTENGFRPYPTGRFAAKVDVFGS
g287      YGAERKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFMENGRFYPSSGRFAAKVDVFGS
300      310      320      330      340      350

410      420      430      440      450      460      469
m287.pep KSVDDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGKFYPAGEEAVAGKYSYR
g287      KSVDDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGFRYPAGEEAVAGKYSYR
360      370      380      390      400      410

470      480      489
m287.pep FTDAEKGFGVFAAGKKEQDX
g287      FTDAEKGFGVFAAGKKDRDX
420      430

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287.seq
1   ATGTTTAAAC  GCAGTGTGAT  TGC AATGGCT  TGTATTGTTG  CCCTTTCAGC
51  CTGTGGGGGC  GCGCGTGGCG  GATCGCCCGA  TGTTAAGTCG  GCGGACAOCG
101 TGTCAAAAAC  TGCGGCCCTC  GTTGTACTAG  AAGATGTCGG  GGAAGAGGTG
151 CTGCCGAAAG  AAAAGAAAGA  TGAGGAGGCG  GTGAGTGGTG  CGCCGCAAGC
201 CGATACGCAG  GACGCAACCG  CGGAAAAGG  CGGTCAAGAT  ATGGCGSGAG
251 TTTCCGCAGA  AAATACAGCG  AATGGCGGTG  CGGCAACAAC  GGATAATCCC
301 GAAATAAAG  ACGAGGGACC  GCAAAATGAT  ATGCGCGAAA  ATGCGCGCGA
351 TACAGATAGT  TGCACACCGA  ATCACACCCC  TGCACCGAAT  ATGCCAACCA
401 GAGATATGCG  AAGACCAAC  CGGATACGCG  GGAATCGGCG  ACAACCGGCA
451 AACCACCGCG  ATATCGGAAA  TCGCGGCGCG  GGAATCGGCG  GGAATCGGCG
501 GTCCGCAGGG  GAAATTCGCG  GCAATACGCG  AGATCAAGCT  GCAATCAAGC
551 CTGAAACCAA  TCAATGTCGC  GGCTCTCAAA  ATCCTGCCTC  TTCAACCAAT
601 CCTAACGCCA  CGAATGCGCG  CAGCGATTTT  GGAAGGATAA  ATGTAGCTAA
651 TGGCATCAAG  CTTGACAGCG  GTTCGGAAAA  TGTACGTTTG  ACACATTGTA
701 AAGCAAAAGT  ATCGCATAGA  GATTTCCTAG  ATGAAGAAGC  ACCACAAAAG
751 TCAGAAATTT  AAAAATTAAG  TGATGAGAAA  AAAATTAATA  AATATAAAAA
801 AGACGAGCAA  CGAGAGAATT  TTGTCGGTTT  GGTTCGTGAC  AGGGTAGAAA

```



- 108 -

a287	KSASSSSARFRRSRRSRLPAEMFLIPVQADTLIVDGEAVSLTGHSNFIAPFEGNYRY	
	300          310          320          330          340          350	
m287.pep	LTYGAEKLPGGSYALRVGVEPAKGEMLAGAAVYNGEVLHFHTENGGRPYPTGRFAAKVDF	
	350          360          370          380          390          400	
a287	LTYGAEKLSGGSYALRVGVEPAKGEMLAGTAYVNGEVLHFMENGRPSPSGGRFPAKVDF	
	360          370          380          390          400          410	
m287.pep	GSKSVVDGIISDGDLMHGTKQFKAAIDGNFGKGTWTENGSGDVSGKFYPGAGEEVAGKYS	
	410          420          430          440          450          460	
a287	GSKSVVDGIISDGDLMHGTKQFKAAVIDGNFGKGTWTEGGGDVSGRFPYGPAGEEVAGKYS	
	420          430          440          450          460          470	
m287.pep	YRPFTAEEKGGFGVFAGKKEQDX	470          480          489
	480          490	
a287	YRPFTAEEKGGFGVFAGKKEQDX	

406

The following partial DNA sequence was identified in *N. meningitidis* <SEO ID 74>:

m06_seq							
1	ATGCACGACG	GCGCTCGTAT	ACCTATCTTT	TTTTCAGTTT	TTAATTATTC		
51	TCGCGTCGGG	ACATACACAG	GTATTCCATC	GCATGCGGGA	GGTTAAACGCT		
101	GTGGCGTGGG	ACAGAAGATT	GTGGCGCGTT	TCGCGAAGAG	TGCGGCTAAA		
151	GACATGATTA	TACAGCGATT	ACACAGCGAG	AAAGTGTGAT	TGTCATATGC		
201	CACATATGGC	CACAGCGATT	CAGGACGATT	GACGAGGGGT	CGTACTACGC		
251	TGTAAGCACT	GATTCGTGGC	GAATACACAA	ACAGCCCTGC	CGTCGCTACC		
301	GATTACACTT	ATCCAGCTAT	CGAAACCCAC	CTGAAAACAA	CATACAGGCG		
351	TTTGACAGCT	ATTACAGCTT	CTTTATCTAC	ACTTTAAGCC	CTGCAGCTAT		
401	CTCGCACCCA	ATCAGACGAT	AGCGAGGAGT	AAAGCAGTCT	GGGCTTAATAT		
451	ATTGCGGGGA	TGGGGGATTA	TGGAATATGA	ACCTGAGCA	CTAACCGCGG		
501	CGACACTGCC	TGTTTCTCCC	ACTTGGTACA	GACGCTTAT	TTCCCTCGGG		
551	GCATAGACGT	TGTTTCTCCT	GCCTAAGCCG	ATACAGATGT	GTTTTATTAAC		
601	ATCGACGTAT	CTGATTCAGT	ACGCACGAG	ACGGAATGCT	ACCTATACAC		
651	CTGCGACGAA	CTGAACACCC	ACCAACAAAT	CGAATATGAT	CTGATGACAA		
701	GAACTATTA	AAATATGCTC	ATCAACCAAC	CGATCAATGA	GTTTTAGACT		
751	CGCTAATAAG	AAATTAACGC	ATTCTGGATG	GGCCCGTATG	AAAGTGAAGA		
801	AGGAATATTA	CCACAGCGAG	GATTAATGGT	CGATTTCTCC	GATCTCCGAC		
851	CATACGCAAA	TCAATCGGGT	AACTCGCCCC	CATCTGGTGA	GGCTGATACG		
901	AGTCATAGG	GGTATGATGA	CACGAGTAA	GTAGTGCAC	AACAATGACA		
951	AGGACACACT	TGA					

This corresponds to the amino acid sequence <SEQ ID 75; ORF 406>:

m406.pep									
1	MQARLLLPIL	FSVFILSAG	TLTIPGSHGG	GKRFAVEQEL	VAASARAARK				
51	DMDLQALHGR	KVALTYATG	DGGGSLTGL	RYSDIALRG	EYNSPAVRT				
101	DYTPYRPTET	AETTSGGTGL	LTTSLSLTNA	PAISRQTSQG	GKSSKSLGN				
151	IGGMDGVRNE	TLTNLPRTA	FLSHLQVTF	PLRGIDVIVP	ANADTVFIN				
201	IVDFGTTIRN	TEMLHYNAT	LKAAQTKYF	AVDRTNKKLL	IKPKNFEAR				
251	AKENYALNM	GPYKXSGIK	PTGELMVDFS	DIRPYGNHTS	NSAPSVFADN				
301	SHBGYSYDSE	VYVRHROGRG	*						

- 109 -

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

**g406.seq**

```

1  ATGCGGGCAC  GGCTGCTGAT  ACCTATTCTT  TTTTCAGTIT  TTATTTTATC
51  CGCCTGCGGG  ACACTGACAG  GTATTCCATC  GCATGGCGGA  GGCAAAACGT
101  TCGCGGTCGA  ACAGGAACCT  GTGGCCGCTT  CTGCCAGAGC  TGCCGTATAA
151  GACATGGATT  TACAGGCATT  ACACGGGACG  AAAGTTGCAT  TGTACATTGC
201  AACTATGGGC  GACCAAGGTT  CAGGCGAGTT  GACAGGGGGT  CGCTACTCCA
251  TTGATGCAC  GATTGCGGCG  GAATACATAA  ACAGCCCTGC  CGTCCGCACC
301  GATTACACCT  ATCCGCGTTA  CGAAACACAC  GCTGAAACAA  CATCAGGCGG
351  TTTGACGGGT  TTAACCACTT  CTTTATCTAC  ACTTAATGCC  CCTGCACTCT
401  CGCGCACCCA  ATCAGACGCT  AGCGGAAGTA  GGAGCAGTCT  GGGCTTAAAT
451  ATTGGCGGGA  TGGGGGATTA  TCGAAATGAA  ACCTTGACGA  CCAACCGCG
501  CGACACTGCC  TTCTTTCCCT  ACTTGGTGCA  GACCGTATT  TTCTTGGCG
551  GCATAGACGT  TGTCTCTCCT  GCCAATGCCG  ATACAGATGT  GTTTATTAA
601  ATCGACGTAT  TCGGAACGAT  ACGCAACAGA  ACCGAATGCG  ACCTATACAA
651  TGCCGAACA  CTGAAGGCC  AAACAAACT  GGAATATTT  CGAGTAGACA
701  GAACCAATA  AAAATTGCTC  ATCAAAACCA  AAACCAATGC  GTTTGAAGCT
751  GCCTATAAG  AAAATTACGC  ATTGTGGATG  GGGCCGTATA  AAGTAAGCAA
801  AGGAATCAA  CGACGGAAG  GATTGATGGT  CGATTCTCC  GATATCCAA
851  CATACGGC  TCATACGGGT  AACTCCGCC  CATCCGTAGA  GGCTGATAC
901  AGTCATGAG  GGTATGGATA  CAGCGATGAA  GCAGTGCAC  AACATAGACA
951  AGGGCACT  TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

**g406.pep**

```

1  MRARLLIPI  FSVFILSACG  TLTGIPSHGG  GKRFVEQEL  VAASARAARK
51  DMDLQALHGR  KVALYIATMG  DQGSGLTGG  RYSIDALIRG  EYINSPAVRT
101  DYTYPREYTT  AETTSGLTGG  LTSSLTLNAP  PALSRTOSSD  GSGRSSGLGN
151  IGGMGDYRNE  TLTINPRDTA  FLSHLVQTVF  FLRGIDVVP  ANADTDVFIN
201  IDVFGTIRN  TEMHLYNAET  LKAQTKLEYP  AVDRINKKLL  IKPKTNAFEA
251  AYKENYALNM  GPYKVSIGIK  PTEGLMVDPS  DIQPYGHTG  NSAPSVEADN
301  SHEGYGYSDE  AVRQHRQGP  *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

**g406/m406**

	10	20	30	40	50	60
g406.pep	MRARLLIPI	FSVFILSACG	TLTGIPSHGG	KRFVEQEL	VAASARAARK	MDLQALHGR
m406	MQARLLIPI	FSVFILSACG	TLTGIPSHGG	KRFVEQEL	VAASARAARK	MDLQALHGR
	10	20	30	40	50	60
g406.pep	KVALYIATMG	DQGSGLTGG	RYSIDALIRG	EYINSPAVRT	DYTYPREYTT	AETTSGLTGG
m406	KVALYIATMG	DQGSGLTGG	RYSIDALIRG	EYINSPAVRT	DYTYPREYTT	AETTSGLTGG
	70	80	90	100	110	120
g406.pep	LTTSSLTLNAP	PALSRTOSSD	GSGRSSGLGN	IGMGDYRNET	TLTINPRDTA	FLSHLVQTVF
m406	LTTSSLTLNAP	PALSRTOSSD	GSGRSSGLGN	IGMGDYRNET	TLTINPRDTA	FLSHLVQTVF
	130	140	150	160	170	180
g406.pep	LTTSSLTLNAP	PALSRTOSSD	GSGRSSGLGN	IGMGDYRNET	TLTINPRDTA	FLSHLVQTVF
m406	LTTSSLTLNAP	PALSRTOSSD	GSGRSSGLGN	IGMGDYRNET	TLTINPRDTA	FLSHLVQTVF
	190	200	210	220	230	240

- 110 -

```

g406.pep      FLRGIDVVS PANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
                |||
m406          FLRGIDVVS PANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
                190      200      210      220      230      240

                250      260      270      280      290      300
g406.pep      IKPKTNAFEAAYKENYALWMGPYKVSIGIKPTEGLMVDFSIDIQPYGNHTGNSAPFSEADN
                |||
m406          IKPKTNAFEAAYKENYALWMGPYKVSIGIKPTEGLMVDFSIDIQPYGNHTGNSAPFSEADN
                250      260      270      280      290      300

                310      320
g406.pep      SHEGYGYSDEAVRQHRQGQPX
                |||
m406          SHEGYGYSDEVVRQHRQGQPX
                310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1  ATGCAAGCAC  GGCIGCTGAT  ACCTATTCTT  TTTTCAGTIT  TTATTTTATC
51  CGCCTGCGGG  ACACATGACAG  GTATTCCATC  GCATGCGCGA  GGTAAACGCT
101  TCGCGGTCTGA  ACAAGAACCT  GTGGCCGCTT  CTGCGCAGAG  TGCCGTTAAA
151  GACATGGATT  TACAGGCATT  ACACGGACGA  AAAGTTGCAT  TGACATTGC
201  AACTATGGGC  GACCAAGGTT  CAGGCGATT  GACAGGGGGT  CGCTACTCCA
251  TTGATGCACT  GATTCTGTGG  GAATACATAA  ACAGCCCTGC  CGTCCGTACC
301  GATTACACCT  ATCCACGITA  CGAATACACC  GCTGAAACAA  CATCAGCGGG
351  TTIGACAGST  TTAACACCT  CTTTATCTAC  ACTTAATGCC  CCGCACTCT
401  CGCGCACCA  ATCAGACCT  ACGGAGATA  AAAGCAGTCT  GGGCTTAAT
451  ATTGCGGGA  TGGGGGATTA  TCGAAGTGA  ACCTTACGA  CTAACCGGG
501  CGACACTGCC  TTCTTTTCCC  ACTTGTGACA  GACCGTATT  TTCTGGGCG
551  GCATAGACGT  TGTTTCTCCT  GCCAATGCC  ATACGGATGT  GTTTATTAA
601  ATCGACGTAT  TCGGAACAGT  ACGCAACAGA  ACCGAATGC  ACCTATACA
651  TGCCGAACAA  CTGAAGCCC  AAACAACCT  GGAATATTTC  GCAGTAGACA
701  GAACCAATTA  AAAATTGCTC  ATCAAAACCA  AAACAATGC  GTTTGAAGCT
751  GCCTATAAAG  AAAATTACGC  ATTGTGGAT  GGACCGTATA  AAGTAAGCAA
801  AGGAATTAAA  CCGACAGAAG  GATTAATGGT  CGATTCTCCT  GATATCCAAC
851  CATACGCCAA  TCATATGGGT  AACTCTGCC  CATCGTAGA  GGCTGATAAC
901  AGTCATGAG  GGTATGGATA  CAGCGATGAA  GCAGTCGAC  GACATAGACA
951  AGGCGAACCT  TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1  MQARLLIPIL  FSVFILSACG  TLTGIPSHGG  GKRFAVEQEL  VAASARAAYK
51  DMDLQALHGR  KVALYIATMG  DQSGSLTGG  RYSIDALIRG  EYINSPAVRT
101  DYTYPYRETT  AETTSGLTGG  LTSLSTLNA  PALSRYSQSD  GSGKSSSLGN
151  IGGMGDYRNE  TLITINPROTA  FLSHLVQTVF  FLRGIDVVS  PANADTDVEIN
201  IDVFGTIRNR  TEMHLYNAET  LKAQTKLEYF  AVDRTNKKLL  IKPKTNAFEA
251  AYKENYALWM  GPYKVSIGIK  PTEGLMVDFS  DIQPYGNHMG  NSAPFSEADN
301  SHEGYGYSDE  AVRRHRQGQP *

m406/a406    ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

                10      20      30      40      50      60
m406.pep      MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAAYKMDLQALHGR
a406          MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAAYKMDLQALHGR
                10      20      30      40      50      60

                70      80      90      100     110     120
m406.pep      KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPYRETTAETTSGLTGG

```



- 111 -

a406	KVALYIATMGDQSGSLTGGYSIDALIRGEYINSPAVRTDYTYPRYEYTTAETTSGLGTG	70	80	90	100	110	120
m406.pep	LTTSLSLTINAPALSRQTSDSGSGSSSLGLNIGGMDGYRNETLTNPRTAFSLHSLVQTVF	130	140	150	160	170	180
a406	LTTSLSLTINAPALSRQTSDSGSGSSSLGLNIGGMDGYRNETLTNPRTAFSLHSLVQTVF	130	140	150	160	170	180
m406.pep	FLRGIDVSPANADTVFINIDVFGTIRNTEMHLYNAETLKAQTKLEYFAVDRTNKKLL	190	200	210	220	230	240
a406	FLRGIDVSPANADTVFINIDVFGTIRNTEMHLYNAETLKAQTKLEYFAVDRTNKKLL	190	200	210	220	230	240
m406.pep	IKPKTNAFEAAKYKENYALWMGPKYVSKGIKFTGELMVDFSDIRFPYGNHMGNSAPSV EADN	250	260	270	280	290	300
a406	IKPKTNAFEAAKYKENYALWMGPKYVSKGIKFTGELMVDFSDIQPYGNHMGNSAPSV EADN	250	260	270	280	290	300
m406.pep	SHREGYGYSDVVRHRRQGQFX	310	320				
a406	SHREGYGYSDVVRHRRQGQFX	310	320				

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

```
m726.seq
1      ATGACCATCT  ATTTCAAAAA  CGGCTTTTAC  GACGACACAT  TGGGCGGCAT
51     CCCCAGAGCG  CGGGTTCGCG  TCCGCGCGCA  AGAATACGCG  GCOCTTTGCC
101    CAGCAGAGCGC  CAGCGCGGCG  CAGATTGCGC  CAGATTACGA  CGCGCGTCGG
151    GTTTTAACCC  CGCGCGGCCG  TCGGCATTAT  CACGAATAGC  ACGCGAAAAA
201    ATGGAAAAAT  AGCAAGACCG  CGCGCGCGCG  CGGTTTCGCG  AACAACAAAA
251    CCGCCTTGCG  ATFTCCGCTC  CGGGAAAACT  CGGACGAACT  CAACACAGCG
301    CTCTCTGCGC  GCTATCCCCA  AGTGAAAAAG  CAGACGTTAT  CAGACGAGCA
351    AAGAAGAGCC  CGTCCGCGCG  AGGGCGCAAA  CAACCGCCGC  ACCCGCATCG
401    TGCGCGAAAT  CGCGCGCGCG  AGGGCGTGA  AATTGACGCT  TTGTATGTAA
451    AAGTATTTCT  AAAAATCGCG  CGCGCTGCTT  GTTTCGCGCG  GCGCATTTAT
501    CGGAAAGCGT  CAGACAGACT  AAGACAAATC  GACACCAATC  CAAACCGCGC
551    CCGGATTGGA  CGCGCTGGAA  AAGGAAATCG  AAGAAATGAC  GCTAAACATC
601    GGCTGA
```

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

```
m726.pep
  1  MTIYFKNGFY DDTLGGIPEG AVAVRAEEYA ALLAGQAGQG QIAADSGRPR
  51  VITPFRPSDY HEWDGKKWKI SKAAAAARFA KQTALAFRL AEKADELKNK
101  LLAGYPQVEI DSFYRQEKEA LARQADNNAP TPLMAQIAAA RGVELDLVLE
151  KVIEKSARLA VAAGAIIGKR QQLEDKLNTI ETAPGLDALE KEISEWTLNI
201  G*
```

The following partial DNA sequence was identified in *N. meningitidis* <SEO ID 82>:

m907-2.seq

1	ATGAGAAAAAC	GCCACGATAC	CCTACCCGTT	AATCTGCAAC	GCCGCCGCCT
51	GTTGTGTGCC	GCCGGTGCGT	TGTTGCTCAG	TCCTCTGGCG	CACGCCGGCG
101	CGTACCGTGA	GGAACACGCT	GCCGACGATT	TGGCTTCGGT	GATGAGGACT

- 112 -

```

151 TCTGTCGGCA GCGTCAATCC GCCGAGGCTG GTGTTTGACA ATCGAAGA
201 GGGCGAGCGT TGGTGTGCTG CCATGTGCGC AGGTTTGCCA AGGTTGCTCC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGTCTG TCAATATCCA GTACGAAGGC
301 AGCGGCGCGG GTTGGGATAC GCAGATTGTG TTGGGCTGA TTGAGGTGA
351 AAGCGGCTTC CGCCAGTATG CAATCAGCGG TGTGGGCGCG CGCGGCTGA
401 TGCAGGTATG CCGCTTTTGG AAAAAGTACA TCGGCAAAAC GCGCACAAAC
451 CTGTTTGGCA TCGGCACCAA CCGTGTGTAC GGCTGTACCA TCCTGGCGCA
501 TTACCGGAAT CTTGAAAAAG GCAACATCGT CCGCGCGCTT CGCGCTTTTA
551 ACAGCAGCTT GGGCAGCAAT AAATATCCGA ACGCCGTTTT GGGCGGCTGG
601 CGCAACCGCT GGCAGTGGCG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

**m907-2 .pep**

```

1 MRKPTDTLPV NLORRRLICA AGALLSLPLA HAGAQRRETL ADDVASVMRS
51 SVGSVNEPRL VFDNPKEGGR WLSAKSARIA RVPFEERERR RLLVNIQVES
101 SRAGLDTQIV LGLIFVESAF RQVAISGVGA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNWLY GCTILRHRYN LEKGNIVRAL ARFNGSLGSN KYFNAVLGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

**m953 .seq**

```

1 ATGAAAAAAA TCATCTTCGC CGCACTCGCA GCCGCCGCCA TCAGTACTGC
51 CTCGCGCGCC ACCTACAAAG TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTCAACACC AGCACCAACG TCGGCGGTTT TTACGGCTCG
151 ACCGGTTCGG TCGAGTTTGA CCAAGCAAAA CGCGCAGGTA AAATCGACAT
201 CACCATCCCC ATTGCCAAC TGCAGGCGGG TTCGAAACAC TTACCGACCC
251 ACCTGAATTC AGCGGACATG TCGATGCGCG CCAATATACC GGACATCCGC
301 TTTGTTTCCA CCAAAATCAA CTTCAACCGC AAAAACTGG TTTCGGTTGA
351 CGGCAACCTG ACCATGCACG GCAAAACCGC CCGCGTCAA CTCAAAGCCG
401 AAAAATTCAA CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTTGTGGC
451 GCGGACTTCA GCACACACAT CGACCAGCAC AAATGGGGCA TGGACTACCT
501 CGTTAACGTT GGTATGACCA AAAGCGTCOG CATCGACATC CAAATCGAGG
551 CAGCCAAACA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

**m953 .pep**

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHENT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIF IANLQSGSQH FTDHLKSADI FDAQYPPDIR
101 FVSTKFNFG KKLVSVDGNI TMHGKAPVK LKAEKENCYQ SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GMTKSVRIDI QLEAAQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

**orf1-1 .seq**

```

1 ATGAAACAA CCGACAACG GACAAACGAA ACACACCGCA AAGCCCGAA
51 AACCGCGCGC ATCCGCTTCT CGCCTGCTTA CTAGCCATA TGCCTGCTGT
101 TCGGCAATCT TCCCAAGCC TGGGCGGGAC ACACTATTAT CGGCACTCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAT AAAGCAAGT TTGCGTGGC
201 GCGCAAGAT ATTGAGGTTT ACAACAAAAA AGGGGAGTTG GTCGGCAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGTTGTC GCGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCGTGG CACATAACGG
351 CGGCTATAAC AAGCTTGATT TTGGTGGCGA AGGAAGAAAT CCGATCAAC
401 ATCGTTTTAC TTATAAAATT GTGAAACGGA ATAATTATA AGCAGGACT
451 AAAGGCCATC CTTATGGCGG CGATTATCAT ATGCCGCGTT TGCATAAAT
501 TGTACAGAT GCAGAACCTG TTGAATGAC CAGTTATATG GATGGCGGA

```

551 AATATATCGA TCAAAATAAT TACCCTGACC GTGTTCTGAT TGGGGCAGGC  
 601 AGGCAATATT GGCAGCTCTGA TGAAGATGAG CCCAATAACC GCGAAAGTTC  
 651 ATATCATATT GCAAGTGGCT ATTTCTGGCT CGTTGGTGGC AATACCTTTG  
 701 CACAAAATGG ATCAGSTGGT GGCACAGTCA ACTTAGSTAG TGAAAAAATT  
 751 AAACATAGCC CATATGGTTT TTATCAACCA GGAGGCTCAT TTGGGCGACG  
 801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAAGCAAAAG TGTTAATTA  
 851 ATGGGGTATT GCAACGSGGC AACCCTATA TAGGAAAAAG CAATGGCTTC  
 901 CAGCTGGTTC TTAAGSATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC  
 951 CCATTTCAGTA TTCTACGAAC CAGCTCAAAA TGGGAAATAC TCTTTTAAAG  
 1001 ACGATAATTA TGGCACAAGA AAATCAATG CCAAACATGA ACGCAATTCT  
 1051 CTGGCTAATA GATTAAAAAC ACGAACGGT CAATTGTTTA ATGTTCTTTT  
 1101 ATCCGACAGA GCAAGAGAAC CTGTTATACA TGCTGCAAGT GGTCTCAACA  
 1151 GTTATCGACC CAGACTGAAT AATGGAGAAA ATATTCTCTT TATTTAGGAA  
 1201 GGAAAAGGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAG  
 1251 ATTATATTTC CAAGGAGATT TTACGCTCTC GCCTGAAAAAT AACGAACTTT  
 1301 GGCAAGGCGC GGGCGTTTCA TACAGTGAAG ACAGTACCCT TACTTGGAAA  
 1351 GTAAACGGCG TGGCAACGCA CCGCTGTCC AAATCGGCA AAGGACGCT  
 1401 GCACGTTCAA GCCAAAGGGG AAACCAAGC CTGATCAGC GTGGGCGAGC  
 1451 GTACAGTCAT TTTGGATCAG CAGGCGAGC ATAAAGGCAA AAAACAAGCC  
 1501 TTTAGTGAAT TCGGCTTGGT CAGGCGGAGC GGTACGGTGC AACTGAATGC  
 1551 CGATAATCAG TTCAACCCCG ACRAACTCTA TTTGCGTTCT CCGGGCGSAC  
 1601 GTTTGGATTT AAACGGGCAT TCGGTTTCGT TCCACCGTAT TCAAAATACC  
 1651 GATGAAGGGG CGATGATTGT CAACCAAAAT CAAGACAAG AATCAACGCT  
 1701 TACCATTACA GGCATAAAG ATATTGCTAC AACCGGCAAT AACACAAGCT  
 1751 TGGATGACAA AAAAGAATTT GCCTACAAGC GTTGGTTTGG CGAGAAAGAT  
 1801 ACGACCAAAA CGAACGGGCG GCCTCAACCT GTTTACGAGC CCGCGCAGA  
 1851 AGACCGCAAC CTGCTGCTTT CCGGCGGAAC AAATTTAAAC SGCAACATCA  
 1901 CGCAACCAAA CGGCAAACTG TTTTTCAGCG GCAGACAAC AC CGCACGCG  
 1951 TACAATCATT TAAACGACCA TTGGTGCRAA AAAGAGGGCA TTCTCGCG  
 2001 GGAATCGTG TGGGACAACG ACTGGATCAA CGCGCAATT AAAGCGGAAA  
 2051 ACTTCCAAT TAAAGGCGGA CAGGCGGTGG TTTCCGCAA TTTTGCAAA  
 2101 GTGAAGGCGC ATTGGCATTT GAGCAATCAC GCCAACAGC TTTTGTGTGT  
 2151 CGCACCGCAT CAAGGCCACA CAATCTGTAC ACGTTCGGAC TGGACGGGTC  
 2201 TGACAAATTG TGTCGAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA  
 2251 TTGACTAAGA CGACATCAG CGGCAATGTC GATCTTGGCG ATCAGGCTCA  
 2301 TTTAATCTC ACAGGGCTTG CCACACTCAA CGGCAATCTT AGTGCAAACT  
 2351 GCGATACAG TTAITACASTC AGCCACAAGC CCAACCAAAA CGGCAACTT  
 2401 AGCCTCGTGG GCAATGCCCA AGCAACATTT AATCAAGCCA CATTAAACGG  
 2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGGCG  
 2501 TACAAAACGG CAGTCTGACG CTTCCTGGCA AGCTAAAGGC AAACGTAAGC  
 2551 CATTCCGCAC TCAAGGTTAA TGTCCTCTTA GCGGTAAGG CAGTATTCCA  
 2601 TTTTGAAGC AGCCGCTTTA CCGGACAAAT CAGGCGGAGC AAGGATACGG  
 2651 CATTACACTT AAAGACAGCG GAATGACGCG TGCGCTGAGC CACGGAAATG  
 2701 GCGAATTTAA ACCTTGACAC CGCCACCTAT GCGCTATCT CCGCTATCG  
 2751 CCAGTATGCG CGAGGGGCGC AAACGCGGAG TGACACAGAT CGCCGCGGCG  
 2801 GCGCTTCGCG CCGTTCGCGC CGTTCCTAT TATCCGTTAC ACGCGCAAT  
 2851 TCGGTAGAAT CCGCTTCAA CAGCTGAGC GTAAAGGCA AATTGAACGG  
 2901 TCAGGGAACA TTCCGCTTTA TGTCGAACT CTTCGCTAC CGCAGGCA  
 2951 AATTGAAGCT GCGGGAAGT TCGGAAGGCA CTTACACCTT GCGGCTCAAC  
 3001 AATACCGGCA ACGAACCTCG AAGCTCGAA CAATTGACGG TAGTGAAGAG  
 3051 AAAAGCAAC AAACCGCTGT CCGGAACCTT TAATTTACCC CTGCAAAAGC  
 3101 AACACGTCGA TGCCGCGCGC TGGGCTTACC AACTCATCG CAAGAACGCG  
 3151 GAGTTCCGCG TGCAATAATC GGTCAAGAA CAAGAGCTTT CCGACAATCT  
 3201 CGCAAGGCA GAAAGCAAAA AACAGGCGGA AAAGACAAC GCGCAAGGCC  
 3251 TTGACGCGCT GATTGCGGCC GGGCGGATG CCGTCGAAA GACAGAAAGC  
 3301 GTTGGCGAAC CGGCCGCA GGCAGGCGGG GAAAATGTG CGATTATGCA  
 3351 GGGGAGGAAA GAGAAAAAAC GGGTCAGCG GGATAAAGAC ACGGCTTTGG  
 3401 CGAAACAGCG CGAAGCGGAA ACCCGGCCGG CTACACCGCG CTTCCCGCG  
 3451 GCGCGCGCGC CCGCGCGGGA TTTGCGGCAA CTGCAACCCC AACCGAGCG  
 3501 CCAACCGCAG CGGCACTGTA TCAGCGGTTA TGCCAATAGC GGTTTAGTG  
 3551 AATTTTCGCG CAGCTCAAC AGCGTTTCG CCGTACAGGA CGAATTAGAC  
 3601 CGCGTATTTG CGGAAGACCG CCGCAACGCC GTTTGGACAA CGGCGATCG  
 3651 GGACACCAAA CACTACCGTT CGGAAGATT CCGCGGCTAC CGGCAACAAA

- 114 -

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3701 CCGACCTGCG CCAATCGGT ATGCAGAAAA ACCTCGGCAG CGGGCGCGTC
3751 GGCATCTGTG TTTCGCACAA CCGGACCGAA AACACCTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCCACGGCGC CGTTTTCGGG CAATACGGCA
3851 TCGACAGGTT CTACATCGCG ATCAGCGCGG GCGCGGTTT TAGCAGCGGC
3901 AGCCTTTCAG AGCGCATCGG AGGCAAAATC CGCCGCGCGG TGCTGCATTA
3951 CGGCATTTCAG GCAGATACCC GCGCGCGTTT CGGCGGATTG GGCATCGAAC
4001 CGCACATCGG CGCAACGCGC TATTTTCGTC AAAAAGCGGA TTACCGCTAC
4051 GAAAAGCTCA ATATCGATAC CCGCGCGCTT GATTCAACG CTAACGCGCG
4101 GGGCATTCAG GAGATCGATC CATTCAAAAC CGCACAACG ATTTTCATCA
4151 CGCTTATATT GAGCTGTGCG TATACGATG CGGCTTCGGG CAAAGTCGA
4201 ACACGCGTCA ATACCGCGCT ATTGCGTCAG GTTTTCGCGA AAACGCGCAG
4251 TGCGCAATCG GCGCTAAACG CCGAANTCAA AGGTTTCAGC CTGTCCCTCC
4301 AGCGTCCGCG CCGCAAGCG CCGCAACTGG AAGCGCAACA CAGCGCGCGG
4351 ATCAAAATTAG GCTACCGCTG GTA

```

This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1-1>:

## orf1-1.pap

```

1 MKTTDKRTE THRKAAPTGR IRFSPAYLAI CLSPGILPCA WAGHTYFGIN
51 YQYYRDFPEN KGFVAVGAKD IEVYNKKGEL VGKSMTKAPM IDFSVVRNG
101 VAAVLVDQYI VSAVHNNGYN NVDFGAEGRN PDQHRFTYKI VKRNNYKAGT
151 KGHYPGGDYH MFLRLKFTVD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG
201 RQYWRSEDEE PNNRESSYHI ASAYSWLWNG NTFQNGSGCG GTVNLGSEKI
251 KHSPYGFLPT GSGFDGSGSP MFIYDAQKQK WLINGVLQTG NPYIKGKNGF
301 QLVRKDFWYD EIFAGDTHSV FYEPQNGKQY SFNDNNGTGG KINAKHEINS
351 LPNRLKTRTV QLFNVSLSET AREPVYHAAG GVNSYRPRIN NGENTSFIDE
401 KGKGLILTSN INCGAGGLYF GGDFTVSPEN NETWQAGVHV ISEDSVTWVK
451 VNGVANDRLS KIGKGLTHVQ AKGENQGSIS VGDGTVILDQ QADDGKKKQA
501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGGRLDLNHG SLSPHRIQNT
551 DEGAMLVNIN QDKESTVTIT GNKDIAITGN NNSLDSKKEI AYNWFGKEDK
601 TTKTNGRLNL VYQFAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTFHA
651 YNHLNDHWSQ KEGIPRGELV WNDNDINRTF KAENFQIKGG QAVVSRINAK
701 VKGDWHLNSH AQAVFGVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL
801 SLVGNACATF NQATLNGNTS ASGNASFNL DHAVQNGSLT LSGNAKANVS
851 HSALNGNVSL ADKAVFHVES SRTGQISGG KDTALHLKDS EWTLPSTGLT
901 GNLNLNDATI TLNSAYRHDA AGAQTGTSATD APRRRSRRSR RSLLSVTPPT
951 SVESRFTLIT VNGKLANGGT FRFMSSELFY RSDKLKLAES SEGTYILAVN
1001 NTGNEPASLE QLTVVEGKDN KPLSENINFT LQNEHVDAGA WRYQLIRKDG
1051 EFRLLHNPKE QELSDKLGA EAKKQAEKDN AQSLDALIAA GRDAVENTES
1101 VAEPARCAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFFR
1151 ARRARRDLPQ LQPQPCPCPC RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VWTSGIRUTK HYRSQDFRAY RQTDLRLQIG MQKNLGSGRV
1251 GILFSHNRT EFPDGIQNS ARLAHAVFG QGTGIDRFYIG ISAGAGFSSG
1301 SLSDIGGGKI RRRVLRIGIG ARYRAGFGG GIEPHIGATR YFVQKADTRY
1351 ENVLNATPGL AFNRYRAGIK ADYSFKFAGH ISITPYLSLS YTDASGKVR
1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAKAG FQLEAGHSAG
1451 IKLGYNW*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

## orf46-2.seq

```

1 TTGGGCATTG CCGCAAAAT ATCCCTTATT CTGTCCATAC TGCGAGTGTG
51 CCGCGCATG CATGCACAG CCTCAGATTG GGCAAGCAT TCTTTTATCC
101 GCGAGGTTCT CGACCGTCAG CATTTCGACG CCGACGGGAA ATACCACCTA
151 TTCGCGAGCA GGGGGGAATC TGCCGAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAATACAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGGCGGCCA
251 TTAAGGAAA TATCGCTAC ATTGTCCGCT TTTCGGATCA CGGGCAGCAA
301 GTCCATTCCC CTTTCGACAA CCATGCCTCA CATTCCGATT CTGATGAAGC
351 CGGTAGTCCC GTTACGGGAT TTAGCCTTCA CCGCATCCAT TCGAGCGGAT

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- 115 -

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401 ACGAACACCA TCCGCCGAC CGCTATGAG GCCACAGGG CGGCGGCTAT
451 CCCGCTCCCA AAGGCGCGAG GGATATATAC AGCTACGACA TAAAGGCGCT
501 TGCCCAAAAT ATCCGCCTCA ACCTGACCGA CACCCGCGAG ACCGGACAC
551 GGCTTGCGGA CGGTTTCCAC AATGCGGTA GTATGCTGAC GCAAGGAGTA
601 GGCGACGGAT TCAAAACGGC CACCCGATAC AGCCCGGAGC TGGACAGATC
651 CGGCAATGCC CCGCAAGCCT TCAACGGCAC TGCAGATATC GTTAAAAACA
701 TCATCGGCGC GGCAGGAGAA ATTGTCGGCG CAGGCGATGC GTGCGAGGGC
751 ATAAGCGAAG GCTCAAAATG CTGTGTCATG CAGGCTTGG GTCTGCTTTT
801 CACCGAAAC AGATGGCGC GCATCAACGA TTTGGCAGAT ATGGCGCAAC
851 TCAGAGACTA TCGCCGACGA GCCATCGCGC ATTGGCGAGT CCAAAACCC
901 AATGCCGCAC AAGCGATAGA ACCGCTCAGC AATATCTTTA TGGCAGGCAT
951 CCCATCAAA GGGATTGGAG CTGTTGCGGG AAAATACGGC TTGGCGCGCA
1001 TCACGGCACA TCCTATCAAG CGGTCCGAGA TGGGCGCAT CGCATTGCGG
1051 AAAGCGAAAT CCGCGCTCAG CGACAATTTT CGCGTAGCGG CATACGCCAA
1101 ATACCCGTCC CTTTACCATT CCCGAARTAT CCGTTCAAAC TTGGAGCAGC
1151 GTTACGGCAA AGAAAACATC ACCTCCTCAA CCGTGCGCC GTCAACCGCG
1201 AAAAATGTCA AACTGCCACA CCAACCCAC CCCAGACAG CGGTACCCCT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACGA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGGCG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAAGCCGAG ATGGGAGGTT GTATAGGAAG TTAATAAAT
1401 GACAACTCGT GAGCAGGTGG AGAAAAATGT TCAGGAATA AGGAACGGTA
1451 ATATAACAG TAACCTTAGC CAACATCCTC AACTAGAGAG GGAATAAAT
1501 AAACATAAAT CTCGCGATGA AATTAATTTT GCAGATGGA TGGGAAAAT
1551 TACCGATAGC ATGAATGACA AGGCTTTTAG TAGGCTTTGT AAATCAGTTA
1601 AAGAGAATGG CTTCACAAA CAGTTTGTGG AGTACGTTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AATAATRTGG GTTTTTGCTG CAGAATACCT
1701 TGGCAGGATA CATGAATTAA AATTTAAAAA AGTTGACTTT CCGTTCTCTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGARTGAATC AGGTAAATGT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

```

orf46-2.pap
1 LGISRKISLI LSLIIVCLEM HAHASDLAND SFIRQVLDLQ HFEFDGKYHL
51 FGSRGELAEK SGHIGLGIQI SHQLGNLMQI QAAIKNIGY IVRFSDHGHE
101 VHSFPDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHFAD GYDGPQGGY
151 PAPKGARDIY SYDIKGVQNI IRLNLTNRS TCQRLADRFH NAGSMLTGGV
201 GDGFKRATRY FELDRSGNA AEAFTNTADI VKNIIIGAGE IVGAGDAVQG
251 TSESGNIAYM HGLGLSTFNI KMARTNDLAD MQLKDYDAAA ATRWAVQNP
301 NRAQCIKAVS NIFMAIPIK IGIVRGKGYV LGGITHAPIK RSQMGIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSNIRNS LEQRYKENI TSSTVPPSHG
401 KNVKLADCRH PKTGVDFDGG GFNFEXHKVK YDRLDIQEL SCGGIPKAPK
451 VFDAPRWEV DRKLNLKLTTR EQVEKNVQRI RGNINNSNF SHAQLEREIN
501 KLKSADEINF ADMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAVIVRGNRR VFAAEYLGR I HELKFKKVDF PVNTSKWNP TDVLNESCNV
601 KRPRYSRK*

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Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

#### Oligonucleotides used for PCR

Table 1

- 116 -

ORF	Primer	Sequence	Restriction sites
279	Forward	CGCGGATCCCATATG-TTGCCTGCAATCAGATT <SEQ ID 90>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTAGAAGCGGCGGCAA <SEQ ID 91>	XhoI
519	Forward	CGCGGATCCCATATG-TTCAAATCCTTTGTCGTCA <SEQ ID 92>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTGGCGTTTGTGCTGC <SEQ ID 93>	XhoI
576	Forward	CGCGGATCCCATATG-GCCGCCCCCGCATCT <SEQ ID 94>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATTACTTTTTGATGTCGAC <SEQ ID 95>	XhoI
919	Forward	CGCGGATCCCATATG-TGCCAAAGCAAGAGCATC <SEQ ID 96>	BamHI-NdeI
	Reverse	CCCGCTCGAG-CGGGCGGTATTCTGGG <SEQ ID 97>	XhoI
121	Forward	CGCGGATCCCATATG-GAAACACAGCTTTACAT <SEQ ID 98>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATAATAATATCCCGCGCCC <SEQ ID 99>	XhoI
128	Forward	CGCGGATCCCATATG-ACTGACAACGCACT <SEQ ID 100>	BamHI-NdeI
	Reverse	CCCGCTCGAG-GACCGCGTTGTGCGAAA <SEQ ID 101>	XhoI
206	Forward	CGCGGATCCCATATG-AAACACCGCCAACCGA <SEQ ID 102>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTCTGTAAAAAAGTATGTGC <SEQ ID 103>	XhoI
287	Forward	CCGGAATTCTAGCTAGC-CTTTCAGCCTGCGGG <SEQ ID 104>	EcoRI-NheI
	Reverse	CCCGCTCGAG-ATCCTGCTCTTTTTTGCC <SEQ ID 105>	XhoI
406	Forward	CGCGGATCCCATATG-TGCGGGACACTGACAG <SEQ ID 106>	BamHI-NdeI
	Reverse	CCCGCTCGAG-AGGTTGTCCTTGTCTATG <SEQ ID 107>	XhoI

## EXAMPLE 2

Expression of ORF 919

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene 919 was cloned in pET vector and expressed in *E. coli*. The product of

protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 3

#### Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

## EXAMPLE 4

Expression of ORF 576

The primer described in Table 1 for ORF 576 was used to locate and clone ORF 576. The predicted gene 576 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B).. These experiments confirm that ORF 576 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 576 are provided in Figure 12. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 576 and the amino acid sequence encoded thereby is provided in Example 1.

## EXAMPLE 5

Expression of ORF 519

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein



- 119 -

and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 6

##### Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene 121 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 7

##### Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene 128 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

- 120 -

Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 8

##### Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worth noting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a reactive band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

- 121 -

antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 9

##### Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 10

##### Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*

- 122 -

*meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example 1.

The foregoing examples are intended to illustrate but not to limit the invention.

- 123 -

### Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

- 124 -

9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.

11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.

12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

15. Nucleic acid encoding a protein according to any one of claims 6-8.

16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.

17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

- 125 -

18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.
19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.
20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.
21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.
22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.
23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.
24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.

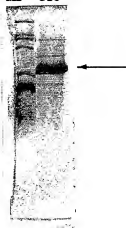
1/18

*FIG. 1A*

919 (46 kDa)

PURIFICATION

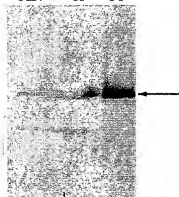
M1 919

*FIG. 1B*

919 (46 kDa)

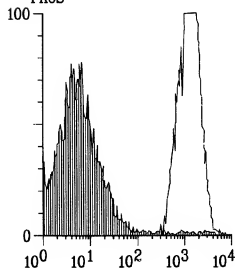
WESTERN BLOT

OMV TP PP

*FIG. 1C*

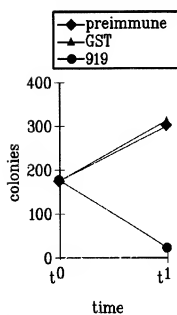
919 (46 kDa)

FACS

*FIG. 1D*

919 (46 kDa)

BACTERICIDAL ASSAY

*FIG. 1E*

919 (46 kDa)

ELISA assay: positive



2/18

*FIG. 2A*

279 (10.5 kDa)

PURIFICATION

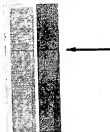
M1 279

*FIG. 2B*

279 (10.5 kDa)

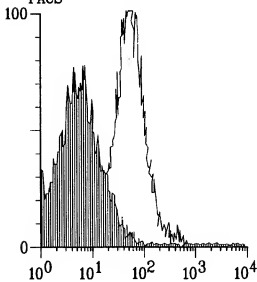
WESTERN BLOT

TP OMV

*FIG. 2C*

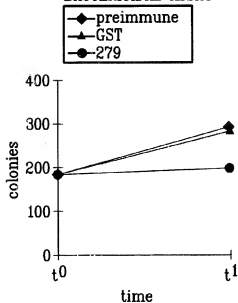
279 (10.5 kDa)

FACS

*FIG. 2D*

279 (10.5 kDa)

BACTERICIDAL ASSAY

*FIG. 2E*

279 (10.5 kDa)

ELISA assay: positive

3/18

*FIG. 3A*

576 (27.8 kDa)

PURIFICATION

M1 576

*FIG. 3B*

576 (27.8 kDa)

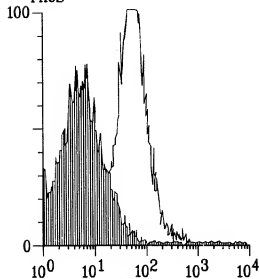
WESTERN BLOT

TP OMV

*FIG. 3C*

576 (27.8 kDa)

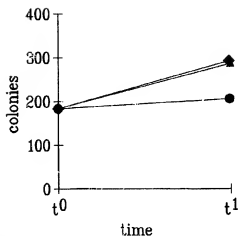
FACS

*FIG. 3D*

576 (27.8 kDa)

BACTERICIDAL ASSAY

◆ preimmune  
▲ GST  
● 576

*FIG. 3E*

576 (27.8 kDa)

ELISA assay: positive

4/18

*FIG. 4A*

519 (33 kDa)

PURIFICATION

M1 519

*FIG. 4B*

519 (33 kDa)

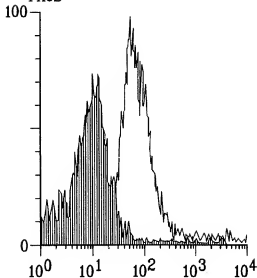
WESTERN BLOT

TP OMV

*FIG. 4C*

519 (33 kDa)

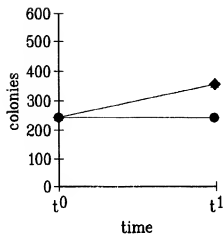
FACS

*FIG. 4D*

519 (33 kDa)

BACTERICIDAL ASSAY

◆ preimmune  
▲ GST  
● 519

*FIG. 4E*

519 (33 kDa)

ELISA assay: positive

5/18

*FIG. 5A*

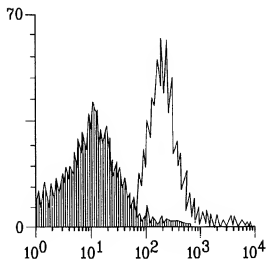
121 (40 kDa)  
PURIFICATION  
M1 121

*FIG. 5B*

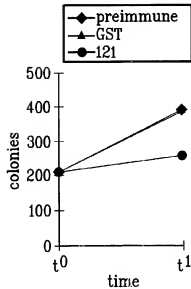
121 (40 kDa)  
WESTERN BLOT  
TP OMV

*FIG. 5C*

121 (40 kDa)  
FACS

*FIG. 5D*

121 (40 kDa)  
BACTERICIDAL ASSAY

*FIG. 5E*

121 (40 kDa)

ELISA assay: positive

6/18

*FIG. 6A*

128 (101 kDa)

PURIFICATION

M1 128

*FIG. 6B*

128 (101 kDa)

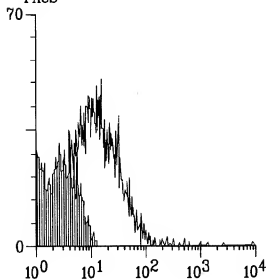
WESTERN BLOT

TP OMV

*FIG. 6C*

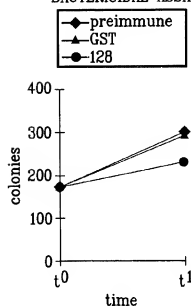
128 (101 kDa)

FACS

*FIG. 6D*

128 (101 kDa)

BACTERICIDAL ASSAY

*FIG. 6E*

128 (101 kDa)

ELISA assay: positive

7/18

*FIG. 7A*

206 (17 kDa)

PURIFICATION

M1 206

*FIG. 7B*

206 (17 kDa)

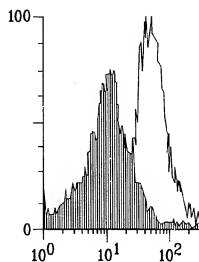
WESTERN BLOT

TP OMV

*FIG. 7C*

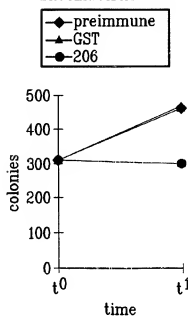
206 (17 kDa)

FACS

*FIG. 7D*

206 (17 kDa)

BACTERICIDAL ASSAY

*FIG. 7E*

206 (17 kDa)

ELISA assay: positive

8/18

*FIG. 8A*

287 (78 kDa)

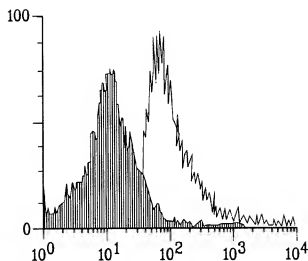
PURIFICATION

M1 287

*FIG. 8B*

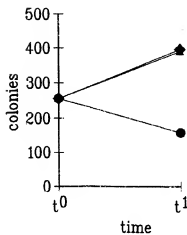
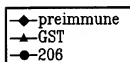
287 (78 kDa)

FACS

*FIG. 8C*

287 (78 kDa)

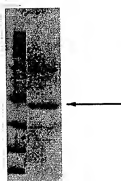
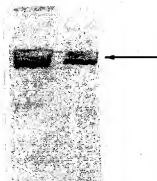
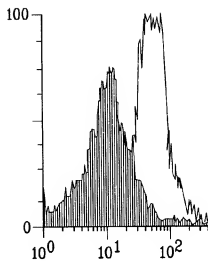
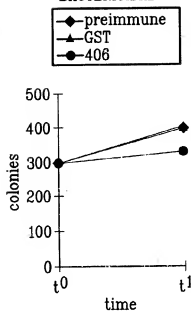
BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)

ELISA assay: positive

9/18

*FIG. 9A*406 (33 kDa)  
PURIFICATION  
M1 406*FIG. 9B*406 (33 kDa)  
WESTERN BLOT  
TP OMV*FIG. 9C*406 (33 kDa)  
FACS*FIG. 9D*406 (33 kDa)  
BACTERICIDAL ASSAY*FIG. 9E*406 (33 kDa)  
ELISA assay: positive



10/18

919

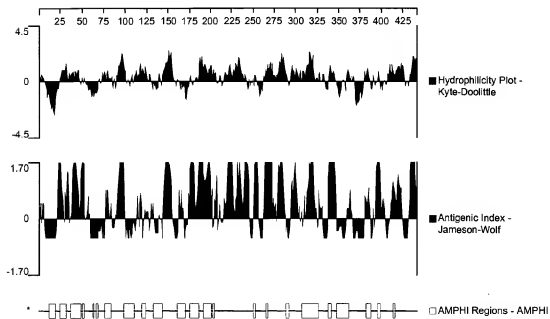
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 10

11/18

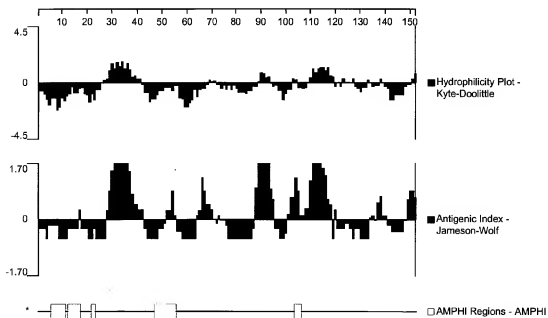
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

12/18

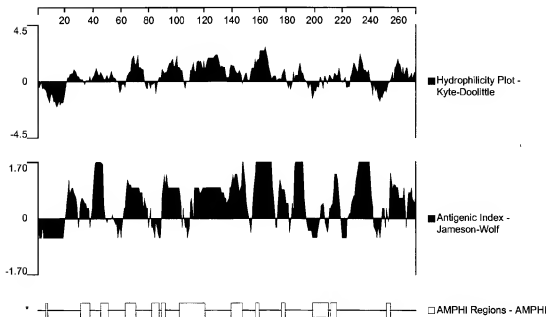
576-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 12

13/18

**519-1**  
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

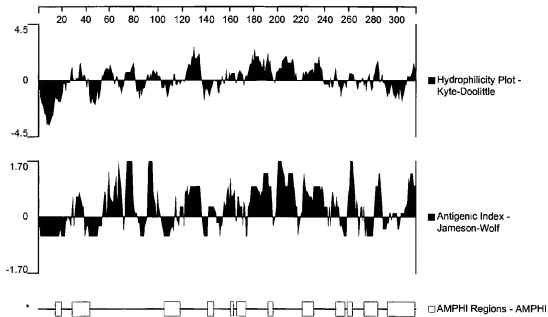


Fig. 13

14/18

**121-1**  
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

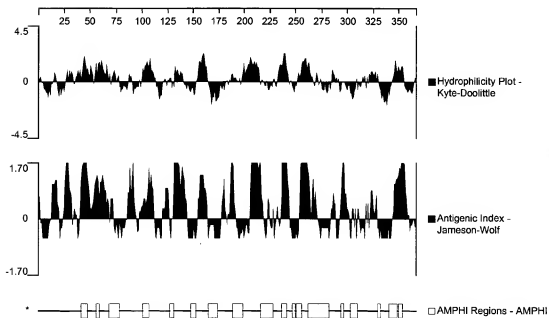


Fig. 14

15/18

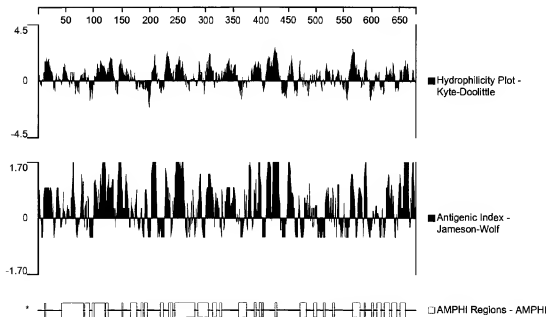
**128-1****Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 15

16/18

206

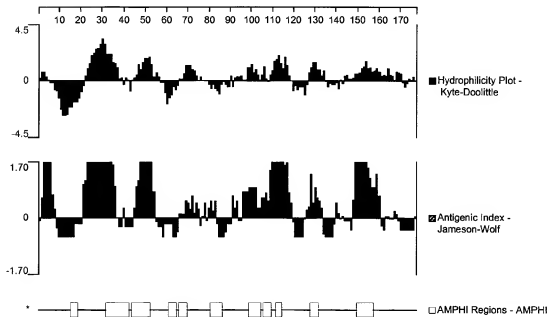
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 16

17/18

287

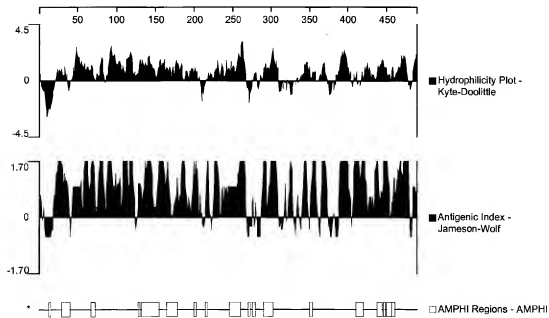
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 17



18/18

406

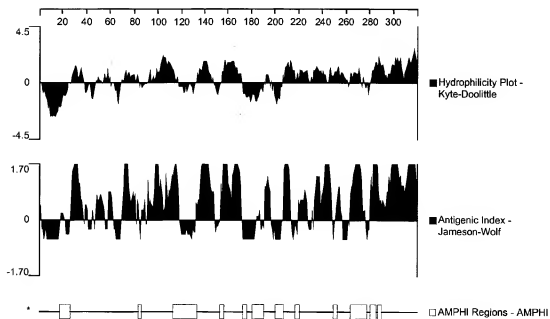
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 18

## Appendix A

-1-

## APPENDIX A

The following DNA sequence was identified in *N. meningitidis* B <SEQ ID NO. 1>:

TAACCTTATCCACATCCAAACGCATAACCGTAAACGATTCACCGTTATGCAANTCTGGC  
 CGCAGACCACTGATTAACATGAGCTTCCATCAGCGCTAAGAAAGCA  
 AAGAAGCTTTATCCGACGAGCGCGCTCCGCGCTATGAGTGGCCGACACGCTCTGCCA  
 ATGCTCTTTCCGCGATATTCAAGTAACAAAGACATCCCCAACCAATATTCATACCGT  
 GGAAACCTTTGCATATCATCGCGCTTGACCGCAGCGCAACCCACAGCATTCGGAAATCA  
 TCCACAGCGCGCAATGCCAGCGCGATCGTGTCTCTCAACACCTGCGTAAATAGGCA  
 TGAATCTTATCCACAGAGCACACGGTTCRAATCGTGCCACTCTCAACACACGCGCTGA  
 ACCAACCTGCGGATATCCGCGCGCTTCAGGCGCTCTAATGAAACAGCATTTGCCACAAAG  
 AGCAATTCATCTCCGTAATCCCGGACGCTCTCTTCCCGCTGCGCGTAAACCGCATTC  
 CAACGATGGTCCAAACGCACTCCGATTTGGCCRAATCTTACGGCTGCGCGGCTTTTGC  
 GCCATTCGTGCAAGGAATTTCCGCTTCCAAACGCGCGATGTCTGCCGTAGCGCTCTCCAAA  
 CGCGCGCGCGCATCTTCCAAATCCGACTGCATCCGATGATTTTCCGTCCAGATGTGTT  
 TGCCTTTGCAATTAAGCGCGGTAAACGGGATTTGGATGCTGAGCAGATTTCTTCAGCATCC  
 CCTGCCCATAGCGTTGTAGAAAAAACACCATCAGAAAAATAAATATTTTTCATTTT  
 AACTTCCATTTAATGCTGTCTGAAGCCGATTTCCGACATCAGACGGCATCGCCACGCG  
 TGTGGATAACTTAAGCGCGGATGGCTTCAACACTTCTCTTTGCGGATTAATGCCAACA  
 CACCTTCACGCTGGCGGTTTTCGCTTACCCACAGAGCAAGGCGAGGCGATGCCGA  
 GTTTGCCCATTTTAATGCTTCTCTGTCGACAGAGGTTTGAAGAGTGTGGATGGCTT  
 CGGCATTCAGTCTTCACGCGCTTCGAGCGCTTCGCAAGCGCAGCATACGGCGCGCGG  
 CTTCATCGTCCGAGTGTTCGTCAGCTGTCTCTCGGACGCGCTTTGTTGACGTAGAAGT  
 AGAAGCACTCGTCGGCAAGCGTCTTCAAGTCTTGGGCGCGTCTTGACAGTCCAAACA  
 CATCTTCCAAAGCGAGTATTTTCGTTTTCATGAATATCCCGCAACGACGAGCGCGCTTGA  
 CGAGTTCGCGAGTTCGCGTGGGTGGTATTTTGAATGTTGCGCGTGTATTCAGTCAATGA  
 GTTTTTTCAGTGTGTTGAGACAGCTGATGATGATGATGATGATGATGATGATGATGATG  
 TGAATTTGCCATTTGTGAAGAAATCATCTGCGCGCTGCGCGCAAGCCAAAGCTGCGAGAT  
 AGTGTAGCATGCTCTCCGCGAGGATGCCCATTTGGCGGAAATCGGTAATGGCAACGAT  
 CGCGCGTCCGTTTGGACATTTTTCGCTTGTCTGTTAAGAAATCATCGGACGATGGCGCT  
 ATTCGCGCAGGTTCGCGTGGATGGCTTTAAGATGTGATTTGTTTTCGCGGTGTGTTCA  
 CATGGTCTCGCGCGGATTAACGTGGGTAAACGCCATGCTGTAGTGTCTACGACACGCG  
 AGAAGTCTGAGTGTGGCGTACCTCGCGCGCGGCGAATTAATCAGTCAATGAGTGTCTGT  
 TGGGATGGAGATTCGCTTGTGACAGTGTCTGCTATTTGGTCAACGCGCAAGCG  
 TTTTGAACGCGCAACGCGTGTGTCAGTCCGACGGGATTTGGCGAGGGTTTACCTACTT  
 CGCGACGCGACGCGCGGTGTAAGTCCGCGAGCGCTCTTTTTCGCGTCTTCTCACCCATCG  
 CTTCAGCGCTCTTCTTGTGCAATGACGATAGTAGGCAATGGCTTTTCTAAAGTTCCG  
 CAATGACCTCTTGTAGCGGTGGAACGGCGAGTTTGTAAACGACGTTGTGCGCGTGT  
 CGTAATGTAGACCGACCCATTTCTCATCGCTCGAGGATGATTTGACGGATTCGCGGTAG  
 AACGCGCGCANGTGGTGTCTTCAATACGTAATAGGAACCTCGCTTTATGATGGCGGCA  
 ACGCCCATGAACAAAGCGCGTGGCAGCGCGCGCGATCTGCACTAGCGCGTGGCGTGG  
 GCGCGAAGCGGTTATGAGGCTCATGAGGCTGCGCGATGCTTGAAGGATTTATTTG  
 TGGTTTACCGCTCTTGGGCATCAAAATGCCCTCGAACCCTGCTCGCGGATAAAGTT  
 CACACGGCATTTTCTTGTGTTTCAATGCTTCGCGACGCGGAACAGTGTATCAGCGCGCG  
 CGACCGAATTCCTTCGGGATTCGCTCCAAAAAAGATTCATGAACACGCTAATTGAAA  
 AATCCGCGCCCATTTTCCAAACGCTAGAGGATTAACGATATCCCTCTTGACGATAA  
 AGATTTTCTTATTTCCGCGATCAACCGCGTGTGTCGCGTGGCAGACATATAAAGCG  
 GGAACCCCAATCTCTCGCATTTTCGCGCGCGCGCAATGGTAGGATTCGTCACAA  
 TCACCGCTGTGACGCAAAACGCGCGCGGATGATGATGATGATGATGATGATGATGATG  
 AAGTGTTCGCGCAAGTCTTCAACACAGATGTTGCGCGCGCGAACCCTCTGTTCAGTG  
 CGTACGCGCGCGCTCGCTCGGCTTCGATATAGCTTTTGTGTCGCGCTCCCGTAA  
 ACACGATTTTGCCTACCTCGCGCTCTGATAAAGTGGATGGCATGTTGTATGCGCTCG  
 GGAACACGAGGAGAGGCGCTTGTGCTCCACGCGCGCGCGCCACACACGCGCGCATCG  
 CCGGACATACGCGCGCAAAACCTGCCACCGCTCGATTAACCGCGCAACCGGATGAGG  
 CAACACCGCAGCAAGGCGCAAAACCTCAACACAGAACCGCGCAACGAGTAAATGCGCA  
 AGCGTTTCGCGGTTTATCTCAATCTCCGCTACACGCTACACGCTACACGCTTACAAATC  
 CGGATCGACATTCAGGAGGACATGATGATGATGATGATGATGATGATGATGATGATGATG  
 GCGCGCACATTTGCGCGCATATCGCGCTGCGCAGACGATGCGCGGATGGAAGACAC  
 ACCCGCAAGCGCTCAATGCTCAAGACCGCGCTACCGATCGTCAACCTTTGCGCGACT  
 TGGTGGCGCGCGTGGCGAAACAGATGCTGCGATGCTCAATGGTCAAAAGCGCAGAA  
 AAGCGCAGCTGATGATGCTCGGATCGCGCTCGACACATCCCAATATCGCAACTTC

## Appendix A

-2-

CTCAAAACAACTCGCTGTTTCTACCCGATTGGCGTTTACACCGGGGCGAACGCGGAAC  
TTTATGAAACACTACGGAACACTCTCGGGGTACTGCCCTTACCGCTGCTGAAGCACGC  
AAATCGGGATACGAGCGACCAATTACGGGGAGGTAAAGAAAAGAGCTGACGACGCC  
GTCAAACTCGGCCATTCAAATGCGCTTAAACGCCGGATGGCGCTGAGCGCGCTTCAGA  
TGGCACTTTTCTTTTCCACCGCTGCGCGGTGCAAACTTATCCACTATCAAAACAGCGC  
GGATCTTTATATCGGCATCGTCTTACCTATTGTTTCAGACGGCATATCCCTGGCGAGCG  
AACGCCCGGAAGAGATATGCGCGCTCTTACAGAGACTTCTATGATCTGTTTCGAAACA  
AGTTTTCAAACATTCGATATCGCGGTTTGAAGCGCTGAAACGCTACGCTTCGAATGAA  
CAAGCGGAATATGATTTATTCGGGGGCACTCGGTTTCGGGCAATCCACATCTCTCAA  
ACTGATTTGGGGCATTTACCAAGCGAGCAGGSGCAAAATCTGTTTACGGGCGAGACTCT  
CGGCACATTTGTCGCAACCAAACTGGCTTTATGCGCCACACATCGGCATCGTGTCCCA  
AGACCACAAAATCCTCTACGACGCGACGCTCTGCAAAACGTCATCTCGCGCTTCGGAT  
TATCGGCTATTCGCGCGCGCAAGCGGAAGCGGTGCGCGCATCGCATCGATGAAAGCTCGG  
CCTGAAGGAGCAGAAATGGAGGATTCGCTAACCTCTCCGGCGGTGAACACCAACGCGCT  
GTGCATCGCGCGCGCGCTGTTTACCAAGCGCGCGCTGCTGATTCGCGGACGACGCTTCGC  
CAACCTCGACGCGCGCTACGGCGTGGATTTATGATGATTTCAAACCTTCCGACGCG  
GGGAACACTACGCTCATGTTGCGGCACTAGCAAGAACTGATGGCGGACTTCGACACCG  
CATCTGCGCGCTCTCGAAGAGGACACTGCACTGAGCATCATCCACTACTCTCGCTGCAC  
GTGCAATCGCGCGCACCGCGCTCAAGCAGCTCTCGCGCAACCGCTTCGCGCACTGCTT  
ACCTCTGATGATGCTCGCGCTCGCATGACCTCGCGCTGTTTATGATCTGGGCATCCAA  
AGCGGGCAAAAGCGTGTGGGCAAACTCAAGAGTTCGCGCAAACTCAAACTATATGGA  
ACCTCGCGCGCAACAAGGAGCAGGATTCGCTCGCGCACTGCTGCGCGCGCAACGCGCT  
GTGCACACATTCGATGATGAGGAGTTCGCTGAGGAGTACGAGCTTCGCTGCTGCTGCTG  
GACCAAAATCTGATTTTCACTGCTTGAAGCGCAACCGCTCGCGGATGCTTTCTGCTTAC  
CGGACCGCGCAACACGCGCGCGCAATTCGAGGCAATCTACGAGACATTAACCAACTG  
CCTATGTTGGAATTCGCGCTCTATGATACCGAATGGGTGCAACCGCTGACCAAACTCAAC  
GAGTTTATCGCAAAATTTTGTGTTTCTTCTCGGACGCTGGGGATGGCTTCTGCTCT  
GTGCGACACAAACCATTCGCGCTGCAATCTCTGACGCGCAAGAGAAATCGAAATCAAC  
AAACTCTTGGGCGCGCGCGGTGTTTATCGCGCGCGCATTCCTTTATCAAGCATGTGG  
CAGAGAGCTTCTTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG  
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TACGCTGGCGGACTGGGCTGGTGTGCGCTTCTGCTATCGCTTGGGCGTATTCGCGCGG  
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## Appendix A

-3-

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## Appendix A

-4-

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## Appendix A

-5-

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## Appendix A

-6-

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-7-

[illegible]



## Appendix A

-8-

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## Appendix A

-9-

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## Appendix A

-10-

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## Appendix A

-11-

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-12-

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-13-

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## Appendix A

-14-

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-15-

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## Appendix A

-16-

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## Appendix A

-17-

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## Appendix A

-18-

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## Appendix A

-19-

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## Appendix A

-20-

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## Appendix A

-21-

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## Appendix A

-22-

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## Appendix A

-23-

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## Appendix A

-24-

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## Appendix A

-25-

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## Appendix A

-27-

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## Appendix A

-28-

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## Appendix A

-29-

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## Appendix A

-30-

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## Appendix A

-31-

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## Appendix A

-32-

[illegible]

## Appendix A

-33-

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## Appendix A

-34-

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-35-

[illegible]



-37-

[illegible]

## Appendix A

-38-

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## Appendix A

-39-

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-40-

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 TTTTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG

## Appendix A

-41-

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-42-

[illegible]

## Appendix A

-43-

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GGGCTGCTTTAAACGCGGCTTCGCGGATTTAGCGGCTGATGCTTCGCGGCTG  
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## Appendix A

-44-

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## Appendix A

-45-

[illegible]

## Appendix A

-46-

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## Appendix A

-47-

[illegible]



## Appendix A

-48-

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## Appendix A

-49-

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## Appendix A

-50-

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## Appendix A

-51-

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## Appendix A

-52-

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## Appendix A

-53-

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## Appendix A

-54-

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## Appendix A

-55-

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## Appendix A

-5-

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## Appendix A

-57-

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## Appendix A

-58-

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## Appendix A

-59-

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## Appendix A

-60-

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## Appendix A

-61-

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-62-

[illegible]

-63-

[illegible]



## Appendix A

-64-

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## Appendix A

-65-

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## Appendix A

-66-

[illegible]

## Appendix A

-67-

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-68-

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## Appendix A

-70-

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## Appendix A

-71-

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## Appendix A

-72-

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## Appendix A

-73-

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## Appendix A

-74-

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## Appendix A

-75-

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-76-

[illegible]

## Appendix A

-77-

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 CGAGGATCCGCTTTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT

-78-

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## Appendix A

-79-

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## Appendix A

-80-

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## Appendix A

-81-

[illegible]

## Appendix A

-82-

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## Appendix A

-83-

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## Appendix A

-84-

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## Appendix A

-85-

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-86-

[illegible]

-87-

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## Appendix A

-88-

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## Appendix A

-89-

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## Appendix A

-90-

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## Appendix A

-92-

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-93-

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## Appendix A

-94-

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-95-

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## Appendix A

-97-

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-98-

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-99-

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## Appendix A

-100-

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## Appendix A

-101-

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## Appendix A

-102-

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## Appendix A

-103-

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## Appendix A

-104-

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## Appendix A

-105-

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